

# **HEMATOPATHOLOGY PEARLS**

# HEMATOPATHOLOGY PEARLS

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*Printed at*

*To*  
*My wife Connie*  
*and*  
*daughter Cecelia*  
*for their continuous*  
*support and encouragement*



## *Preface*

---

I started this book when I was a junior faculty member as a note to residents outlining the most important key information in hematology. Multiple textbooks, lecture notes, as well as my own practice experience have been used in assembling this book. This book is not intended to be a textbook for pathologists. Rather, it is an overview and study guide for medical students, residents and fellows to use during their training and preparation for board examinations.

I would like to thank my mentor, Dr Ivan Damjanov, whose encouragement, guidance and support enabled me to finish this book. I would also like to thank Dr Mark Davis, Dr Jamie Boone and Dr Sarah McHugh who helped proofread this manuscript during their busy residency training and Mr Dennis Friesen who helped me prepare the illustrations.

**Da Zhang**

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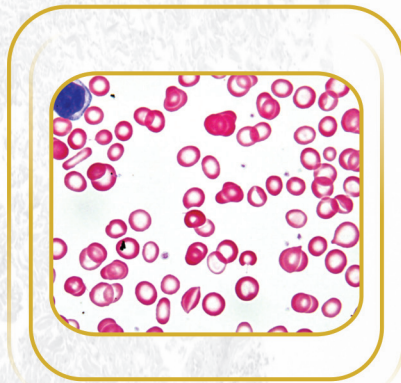
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CHAPTER

1

# Hematopoiesis and Hematology Testing



### *Hematopoiesis and Blood Cells*

---

Hematopoietic stem cells are self-renewing cells that differentiate and become committed to different cell lineage.

Pluripotent stem cells and early progenitor cells can be cultured on culture media. In culture media, progenitor cells are defined as “colony forming units” (CFUs); CFUs are earliest detectable progenitor cells that give rise to **granulocytes**, **erythroblasts**, **monocytes** and **megakaryocytes**, which together are termed CFU<sub>GEMM</sub> or CMP (common myeloid progenitor). The more mature and specialized precursor cells are termed CFU<sub>GM</sub> (**granulocytes** and **monocytes**), CFU<sub>E</sub> (**erythroblasts**), and CFU<sub>EO</sub> (**eosinophil**). The burst forming units (BFU<sub>E</sub>) of the erythroid lineage are early progenitor cells committed to erythrocyte differentiation and are the early ancestors of the CFU<sub>E</sub>. The BFU<sub>E</sub> has a limited capacity of proliferation and gives rise only to erythrocyte colonies. BFU<sub>E</sub> is insensitive to erythropoietin, and its progeny must go through as many as 12 divisions before they become mature erythrocytes.

The proliferation of stem cells and progenitor cells is under the control of cytokines. IL-3 and GM-CSF are non-lineage specific cytokines, which act on pluripotent and early progenitor cells; they are required for self-renewal and differentiation throughout hematopoietic process. In contrast, cytokines such as Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), IL-5, thrombopoietin and erythropoietin all act on more mature and specific hematopoietic lineage cells. Erythropoietin, a glycosylated peptide with its gene located on the chromosome 7, is predominantly derived from the kidney and with a small amount from the liver. It stimulates erythropoiesis at the stage of CFU<sub>E</sub>. Thrombopoietin, a polypeptide with its gene located on chromosome 3, is mainly formed in the liver and small portion in the kidney. It stimulates the formation of megakaryocytes and release of platelets. It also works with erythropoietin to stimulate erythroid progenitor cells. The elimination half-life of thrombopoietin is approximately 30 hours. It is the longest half-life of the hematopoietic growth factors.

### **The Sequence of Hematopoietic Cell Maturation**

- 1. Erythroid lineage:** Pluripotential stem cell → myeloid stem cell → BFU<sub>E</sub> → CFU<sub>E</sub> → pronormoblast → basophilic normoblast → polychromatic normoblast → orthochromic normoblast → polychromatic erythrocyte (reticulocyte) → erythrocyte.

The **last stage of erythroid lineage**, which is still capable of division, is the **orthochromic** cell stage.

2. **Megakaryocyte lineage:** Pluripotential stem cell → myeloid stem cell → CFU<sub>GEMM</sub> → CFU<sub>MEG</sub> → megakaryoblast → megakaryocyte → platelets.
3. **Myeloid lineage:** Pluripotential stem cell → myeloid stem cell → CFU<sub>GEMM</sub> → CFU<sub>GM</sub> → myeloblast → promyelocyte → myelocyte → metamyelocyte → band → polymorphonuclear neutrophil.

Monocyte maturation follows the following sequence: from CFU<sub>GM</sub> monoblast → promonocyte → monocyte then moves to circulation and tissue.

The **last stage of myeloid lineage**, which is still capable of division, is the **myelocyte** stage. The earliest detectable, specific myeloid antigen is **CD33**.

4. **Eosinophil:** Pluripotential stem cell → myeloid stem cell → CFU<sub>GEMM</sub> → CFU<sub>EO</sub> → CFU<sub>GM</sub> → myeloblast → promyelocyte → eosinophilic myelocyte → eosinophilic metamyelocyte → eosinophilic band → eosinophil.
5. **Basophil:** Pluripotential stem cell → myeloid stem cell → CFU<sub>GEMM</sub> → CFU<sub>BASO</sub> → myeloblast → promyelocyte → basophilic myelocyte → basophilic metamyelocyte → basophilic band → basophil → mast cell.
6. **B-cell:** Pluripotential stem cell → lymphoid stem cell → pre-B → B-lymphoblast → B-prolymphocyte → B-lymphocyte → plasma cell.  
**Cytoplasmic CD22**, CD10, CD19, TdT and HLA-DR are present on the very early B-cells.
7. **T-cell:** Pluripotential stem cell → lymphoid stem cell → pre-T → T-lymphoblast → T-prolymphocyte → T-lymphocyte.

**CD7** is the earliest antigen present on the T-cell surface, and **cCD3** (cytoplasmic CD3) is the earliest T-cell lineage specific antigen in the cytoplasm.

## Erythrocytes

The main function of red blood cell is to carry oxygen to tissues and return carbon dioxide to the lung. Hemoglobin molecules present in the RBC contain four peptide chains. The lifespan of a normal RBC is 120 days.

When the oxygen is released, the hemoglobin  $\beta$ -chains are pulled apart so that 2,3 DPG can move in and lower the affinity of hemoglobin for oxygen, resulting in improved delivery of oxygen to the tissue.

**TABLE  
1-1**
**Comparison of embryo, newborn and normal adult hemoglobin**

Hemoglobin	Peptide chain	Site of erythropoiesis
<b>Embryo</b> Gower <sub>1</sub> Portland Gower <sub>2</sub>	(2ζ2ε) (2ζ2γ) (2α2ε)	Yolk sac ζ is equivalent to the α chain ε is equivalent to the β chain
<b>Newborn</b> HbA HbA <sub>2</sub> HbF	(2α2β) (2α2δ) (2α2γ)	Bone marrow and spleen
<b>Normal adult</b> HbA HbA <sub>2</sub> HbF	(2α2β) (2α2δ) (2α2γ)	Bone marrow

Hemoglobin affinity for oxygen is increased by HbF, increased pH and decreased level of 2,3 DPG. As the affinity for oxygen increases, the disassociation curve will shift to the left.

Hemoglobin affinity is decreased by HbS, decreased pH, increased level of 2,3 DPG. As the affinity for oxygen decreases, the disassociation curve will shift to the right. The site of erythropoiesis is listed in Table 1-1.

## Neutrophils

Neutrophil production and differentiation in the bone marrow takes 6 to 10 days. Large numbers of band and segmented neutrophils are stored in the bone marrow as a reserve pool (10-15 times of the peripheral neutrophils). After being released from the bone marrow, neutrophils typically spend 6-12 hours in the peripheral blood circulation before migrating into tissues. Neutrophils then survive about 2-4 days in the tissue before being destroyed. A prominent characteristic of neutrophils is the abundance of cytoplasmic granules. The granules function as intracellular stores of proteinase that associate with neutrophils' adhesion, migration, phagocytosis, and killing of microorganisms. The neutrophils' granules are listed in Table 1-2.

### *The Left and Right Shift of Granulocytes*

The degree of nuclear lobulation of polymorphonuclear neutrophils (PMNs) is an indication of the cell's maturity. A predominance of hypolobulated cells is called a **"left shift"**. Conversely, a predominance of cells with four nuclear



**TABLE  
1-2****Granule contents of neutrophils**

Primary (azurophilic) granules	Specific granules
Myeloperoxidase Serine protease Acid hydrolase Lysozyme (muramidase, etc.)	Lysozyme (muramidase, etc.) Collagenase Gelatinase

lobes is called a “**right shift**”. For practical purposes, a left shift is usually noted when more than 10–12% bands are seen on the CBC differential count, or when the total PMN count (segmented and band forms) is more than 80%.

**Potential causes of left shift:** Bacterial infection, toxemia, hemorrhage, and myeloproliferative disorders.

**Potential causes of right shift:** Liver disease, megaloblastic anemia, iron deficiency anemia, glucocorticoid use, and reaction to stress.

## Eosinophils

Eosinophils have receptors for IgE, histamine, the Fc portion of immunoglobulin, and complement. They are capable of phagocytosis. Their granules are membrane bound organelles with a “crystalloid” core. Eosinophils are particularly important in allergic and parasitic disease. Eosinophils release arylsulfatase and histaminase, which inactive histamine and SRS-A released from mast cells.

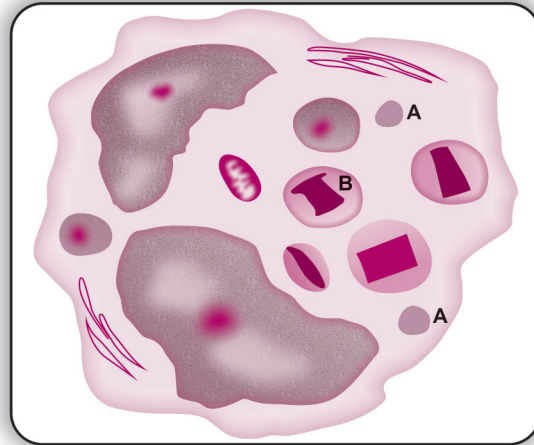
A major constituent of eosinophil is the **Charcot-Leyden protein**, which is composed of **lysophospholipase**. Massive infiltration of eosinophils often leads to disintegration of cells and formation of **Charcot-Leyden crystals**. Charcot-Leyden crystals are bipyramidal, hexagonal crystals that may be seen in tissue and fluid specimen (Fig. 1-1 and Table 1-3).

## Basophils and Mast Cells

They are both derived from the bone marrow; however, their relationship is not entirely clear.

Mature and immature basophils both contain coarse, densely stained blue-black granules with scattered red-purple granules of varying shapes and sizes.





**Fig. 1-1:** Diagram of the ultrastructure of an eosinophil.

- A. Primary granules contain Charcot-Leyden crystals.
- B. Rectangular amorphous crystalloid bar in secondary granules contain major basic protein, eosinophil peroxidase, and other secondary/special granules.

**TABLE  
1-3**

**Granule contents of eosinophils**

Primary granules	Secondary/special granules
Charcot-Leyden crystal (lysophospholipase) Eosinophil peroxidase	Eosinophil peroxidase Major basic protein (forms the crystalline core of the granules) Eosinophil cationic protein Eosinophil-derived neurotoxin (aka eosinophil protein X) Gelatinase

The mast cells are also referred as tissue basophils.

Mast cells are larger than basophils, contain black, bluish-black, or reddish-purple metachromatic granules. The granules may overlay the cytoplasm and obscure the nuclear borders (Table 1-4).

## Monocytes

Monocytes share a common stem cell origin with myeloid cells. Mature monocytes have gray-blue cytoplasm and indented nuclei. The cytoplasm may contain vacuoles or fine granules. Monocytes spend only a short time in the bone marrow. After circulating in the blood for 12-24 hours, monocytes

**TABLE  
1-4****Comparison of basophils and mast cells**

	<b>Basophils</b>	<b>Mast cells</b>
Size	10-15 $\mu\text{m}$	15-30 $\mu\text{m}$
Mitotic potential	No	Yes
Location	Blood or bone marrow	Tissue or bone marrow
Nuclear	Round	Round in earlier stage and segmented in later stage
Cytoplasm	Moderate amount	Abundant granules
Cytochemistry	Chloracetate esterase (+)	Myeloperoxidase (+)
Granule color	Blue-black, some purple to red	Black, bluish-black, or reddish-purple
Granule content	Both contain heparin, SRS-A, histamine, tryptase	

migrate into the tissue as antigen presenting cells (APC) or macrophages without dividing.

### T-lymphocytes

Mature T-cells comprise 65-80% of the circulating lymphocytes in peripheral blood. There are two major subsets, CD8+ suppressor (aka cytotoxic) T-cells (predominantly in bone marrow), and CD4+ helper T-cells (predominantly in peripheral blood).

Maturation of T-cells occurs in the thymus. T-cells that have migrated from the bone marrow begin the thymic maturation process in the subcapsular region of the lobules of the thymus. As they mature, T-cells progress inward from the subcapsular region, to the cortical region, and finally the medullary region where they are released into the peripheral blood. As the T-cells mature, cytoplasmic CD3, TdT, and CD7 are expressed first, followed by CD2, and then CD5. When the maturing T-cells have reached the cortex region, they express CD1a and co-express CD4/CD8. When they have reached full maturity in the medullary region, the T-cells express surface CD3, and either CD4 or CD8. Rearrangement of the T-cell receptor (TCR) occurs in a specific sequence following the order of  $\delta$ ,  $\gamma$ ,  $\beta$ , and  $\alpha$ . The delta ( $\delta$ ) gene (14q11) is rearranged first, followed by rearrangement of the gamma ( $\gamma$ ) gene (7p14). This leads to expression of  $\gamma/\delta$  T-cells (a minority of the circulating T-cell population), or in the case of the majority of T-cells, the T-cells progress on to rearrange the beta ( $\beta$ ) gene (7q34), followed by deletion of the delta gene, and finally, rearrangement of the alpha ( $\alpha$ ) gene (14q11) to produce  $\alpha/\beta$  T-cells (95% of the circulating T-cell population).

### B-lymphocytes

B-cells comprise 5-15% of the circulating lymphocyte population in the peripheral blood. B-cells express CD10, CD19, CD20, CD22, CD79a, and HLA-DR. Plasma cells are mature B cells that secrete immunoglobulin.

B-cell maturation occurs in the bone marrow and follows a sequential B-cell gene rearrangement process. The first step in the production of a functional immunoglobulin involves recombination at the heavy chain locus (IgH) on chromosome 14q32 by joining a diversity (D) segment with a joining (J) segment to form a D-J fusion, followed by joining a IgH variable (V) region to the D-J fusion to form VDJ fusion. Following rearrangement of IgH, the next rearrangement is the light chain. The kappa light chain (located on chromosome 2p12) rearrangement occurs first, if unsuccessful, then lambda light chain (located on chromosome 22q11) rearrangement occurs.

### Natural Killer Cells (NK Cells)

NK cells are minor population of cells, which do not carry either T- or B-cell markers. Large granular lymphocytes seen in peripheral blood smears comprise a major proportion of NK cells. Interferon- $\gamma$  and IL-2 stimulate NK cell proliferation.

## *Hematology Testing*

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### Anticoagulant Agents Used for Peripheral Blood Cell Counts

1. EDTA is the anticoagulant of choice. EDTA can cause platelet clumping and/or satellitism in automated blood counters which is referred to as EDTA-pseudothrombocytopenia. EDTA is also the preferred anticoagulant agent for the extraction of DNA from plasma.
2. Citrate causes dilution of the specimen, which requires mathematical correction.
3. Heparin may cause WBC and platelet clumping; and therefore is unsatisfactory as an anticoagulant.

### Terminology and Important Calculations

The hematology analyzer is a laboratory instrument that generates a histogram showing size distribution on the X-axis and relative number of particles on

**TABLE  
1-5****Comparison of MCV, RDW and their corresponds diseases**

MCV (fL)	Normal RDW	High RDW
<70	Thalassemia Anemia of chronic disease	Iron deficiency HbH
Normal	Anemia of chronic disease Hereditary spherocytosis Bleeding	Early or partially treated iron or Vitamin D deficiency Sickle cell disease
>100	Aplastic anemia Myelodysplastic syndrome	B <sub>12</sub> or folic acid deficiency

the Y-axis. RBC and MCV are directly measures. MCHC and MCH are calculated values (Table 1-5).

Spurious analyzer readings may occur. The causes of spuriously increased WBC count include cryoglobulin, Heparin, monoclonal protein, nucleated red cells, platelet clumping, and unlysed red cells. The causes of spuriously decreased WBC count include clotting and fragmented WBC.

- 1. RBC (red blood cell):** The normal reference range for males is  $4.6-6.0 \times 10^6/\mu\text{L}$ , and for females is  $4.1-5.4 \times 10^6/\mu\text{L}$ . The presence of cryoglobulin, a WBC count  $>50,000/\mu\text{L}$ , or large platelets may lead to spuriously increased RBC count. Microcytic red blood cells (schistocytes, iron deficiency anemia, thalassemia) clotting, or agglutination may lead to spuriously decreased RBC counts.
- 2. MCV (mean corpuscular volume):** The normal reference range is 80-100 fL. It is a measurement of the red blood cell volume or size. In cases of red blood cell clumping (warm or cold agglutinins), or osmotic abnormalities (hyperglycemia, hypernatremia), the MCV may spuriously elevated.
- 3. RDW (red blood cell distribution width):** The normal reference range is 11.5-14.5%. It measures anisocytosis (difference in cell size). An increase RDW may indicate a mixed cell population.

RDW is calculated as following:

$$\text{RDW} = \frac{\text{Standard derivation of MCV}}{\text{MCV}} \times 100$$

- 4. Hb (hemoglobin concentration):** The normal reference range for males is 14-18 g/dL, and for females is 12-16 g/dL. A commonly used method

is convert hemoglobin to cyanhemoglobin and then measure the absorbance at a wavelength of **540 nm**.

Hgb is measured spectrophotometrically; therefore, increased sample turbidity such as paraprotein, lipids, abnormal hemoglobins, or nucleated cells can lead to erroneous results.

- 5. Hct (hematocrit):** The normal reference range for males is 40-50%, and for females is 37-47%. It is the percentage of blood volume occupied by RBCs. Errors can occur in centrifugation due to plasma trapping, over dilution by anticoagulant, or by prolonged tourniquet application. The hematocrit is calculated as following:

$$\text{Hct} = \text{RBC number} \times \text{MCV}$$

- 6. MCH (mean corpuscular hemoglobin):** The normal reference range is 27-31 pg. It is the average amount of hemoglobin per cell. The MCH is calculated as follows:

$$\text{MCH (pg/cells)} = \frac{\text{Hb (g/L)}}{\text{RBC (cells/L)}}$$

**MCHC (mean corpuscular hemoglobin concentration):** The normal reference range is 32-36 g/dL. MCHC is increased in spherocytosis and decreased in microcytic or hypochromic anemia. Since this is a calculated value, an erroneous value for hemoglobin may affect the MCHC value.

MCHC is calculated as follows:

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL)}}{\text{Hct (L/L)}} \times 100$$

- 7. Reticulocyte count** used as a general indicator of bone marrow erythropoiesis and release. A normal or decreased reticulocyte count associated with moderate or marked anemia is strong evidence that the bone marrow is not responding appropriately. An increased reticulocyte count indicates a rapid erythroid cells turnover, which may be associated with blood loss, or acute/chronic hemolysis. The life span of reticulocytes is approximately 3-4 days in the bone marrow and 24 hours in peripheral blood.

Reticulocytes cannot be visualized with the regular Giemsa stain, therefore special stains such as new methylene blue and brilliant cresol blue are used to precipitate the residual RNA material in these cells to visualize reticulocytes. For automated or flow cytometry analysis, reticulocytes can be stained with auramine O and thiazole orange.

The **corrected reticulocytes count (CRC)** is calculated as follows:

$$\text{CRC} = \frac{\% \text{ reticulocytes} \times \text{Hct}}{45}$$

The reticulocyte production index (RPI) is calculated as following:

$$\text{RPI} = \frac{(\% \text{ reticulocytes} \times \text{Hct})}{45} \times \frac{1}{\text{correction factor}}$$

Correction factor

Hct	Correction factor
40-45	1.0
35-39	1.5
25-34	2.0
15-24	2.5
<15	3.0

The normal range of RPI is 1 to 2. In an anemic patient,  $\text{RPI} < 1$  indicates a decreased production of reticulocytes and red blood cells.  $\text{RPI} > 2$  indicates an increased production of reticulocytes to compensate loss of red blood cells (destruction or bleeding).

8. **Serum iron** measures iron bounded to transferrin (ferric form). There is diurnal variation (highest level in the morning); therefore, serum iron levels should be drawn in the morning.
9. **Total iron bounding capacity (TIBC)** measures the concentration of transferrin. It indicates the iron concentration needed to saturate all transferrin-binding sites.

The normal percentage of transferrin saturation (PTS) is 30% and is calculated as following:

$$\text{PTS} (\%) = \frac{\text{Serum iron (mol/L)}}{\text{TIBC (mmol/L)}} \times 100$$

10. **Serum ferritin:** Ferritin is a storage complex of apoferritin and iron. It is a relatively sensitive and reliable indicator for iron deficiency anemia. It is elevated in iron overload (sideroblastic anemia, hemochromatosis), Gaucher's disease, and inflammatory diseases (ferritin is also an acute phase reactant). If iron deficiency and inflammatory disease co-exist, the serum ferritin level may be normal.
11. **Soluble serum transferrin receptor (STFR):** The transferrin receptor acts as an iron-transporting molecule, it present on most cell surface. The expression of transferrin receptors are depended on the concentration

## 12 Hematopathology Pearls

of iron in the cellular cytoplasm. STFR is increased in iron deficiency anemia and hemolytic anemia

12. **Free erythrocyte protoporphyrin (FEP):** FEP is useful in distinguishing iron deficiency anemia and thalassemia minor. FEP is elevated when there is a failure of iron incorporation to heme (iron deficiency, sideroblastic anemia, anemia of chronic disease, lead poisoning). Thalassemia is associated with abnormal hemoglobin synthesis, but not abnormal heme synthesis, so the FEP level is normal.
13. **HPLC (high performance liquid chromatography):** HPLC measures HbA<sub>2</sub> (increased in most  $\beta$ -thalassemia) and globin chain ratio.
14. **Interfering substances of automated hematology analyzer (Table 1-6):** Check for hemolysis and clotting before use the specimen.

**TABLE  
1-6**

**Potential causes of erroneous results of automated hematology analyzer**

	<b>Falsely increase</b>	<b>Falsely decrease</b>
WBC	Cryoglobulin Extremely elevated protein Nucleated RBC Unlysed RBC Malaria parasites in RBC Platelet clumping	WBC aggregates Fragmented WBC
RBC	Cryoglobulin Giant platelets High WBC count	Autoagglutination Microcytic RBC
Platelet	Cryoglobulin Microcytic RBC Fragmented WBC	Giant platelets Platelet clumping Platelet satellitosis
Hemoglobin	Cryoglobulin High WBC count Severe lipidemia Heparin	
MCV	Autoagglutination Hyperglycemia High WBC count	Cryoglobulin Giant platelets

# Disorders of Red Blood Cells





**TABLE  
2-1****Red blood cell morphology (peripheral blood smear)**

Anisocytosis (Fig. 2-1)	Red blood cells are of unequal <b>size</b> . RDW is a quantitative measure of the degree of anisocytosis.
Poikilocytosis (Fig. 2-2)	Red blood cells of abnormal <b>shape</b>
Acanthocyte (spur cell, Fig. 2-3)	Irregular spiculated cells with projections of varying length due to altered blood cell membrane lipids  Associated with: Abetalipoproteinemia Severe liver disease Splenectomy Hypothyroidism Vitamin E deficiency
Autoagglutination (Fig. 2-4)	Clumping of RBCs, outline of individual cells may not be evident.  Associated with: Antigen/antibody reaction Infections (mycoplasma, EBV) Lymphoma Autoimmune hemolytic anemia
Basophilic stippling (Fig. 2-5)	Punctate basophilic inclusions due to precipitated RNA.  Associated with: Heavy metal poisoning (lead, zinc, arsenic silver, mercury) Thalassemia MDS Severe megaloblastic anemia Pyrimidine 5'-nucleotidase deficiency Congenital dyserythropoietic anemia
Bite cell (Fig. 2-6)	Smooth semicircular defect (Heinz body pitting by spleen).  Associated with: G6PD deficiency Drug-induced hemolysis.
Cabot's ring (Fig. 2-7)	Red-purple staining thread-like filaments in the shape of a ring or figure 8 in stained red blood cell, often in association with basophilic stippling. They may also appear as granules in a linear array rather than as complete rings. Cabot rings are considering remnants of microtubules from a mitotic spindle or remnants of the nuclear membrane.

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Dimorphic RBC population (Fig. 2-8)	Two morphological distinct population of RBCs  Associated with: RBC transfusion MDS Vitamin B <sub>12</sub> deficiency Folate deficiency Iron deficiency
Echinocyte (Burr cell, Fig. 2-9)	Short evenly spaced spicules with preserved central pallor due to altered red blood cell membrane lipid.  Associated with: Renal disease (Uremia) Pyruvate kinase deficiency (PKD) Microangiopathic hemolytic anemia Artifact
Heinz body	Deposits of precipitated, denatured hemoglobin. Crystal violet or brilliant cresyl blue stains are used to visualize these cytoplasmic inclusions.  Associated with: Oxidative stress including G6PD deficiency (usually contains 3-4 Heinz body/cell compared to normal which usually has one cell) Drugs and toxins Unstable hemoglobins
Hemoglobin C crystal (Fig. 2-10)	Dark red, hexagonal-shaped crystal  Associated with: Homozygous hemoglobin C disease
Hemoglobin SC crystal (Fig. 2-11)	Dark red, with 1-2 finger-like projections may look like a mitten.  Associated with: Hemoglobin SC disease
Howell-Jolly body (Fig. 2-12)	Small basophilic cytoplasmic inclusion and is a DNA nuclear remnant.  Associated with: Postsplenectomy Hemolytic disease Megaloblastic anemia
Elliptocyte (Fig. 2-13)	Elliptical shape due to abnormal cytoskeletal protein  Associated with: Hereditary elliptocytosis

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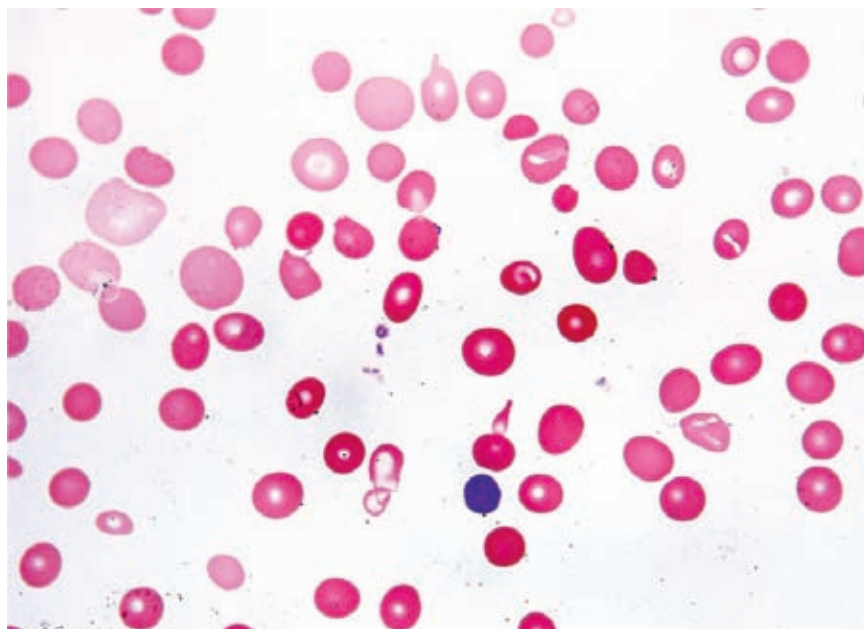
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Pappenheimer body (Fig. 2-14)	<p>Small dense basophilic granules due to iron-containing mitochondrial remnants or siderosome.</p> <p>Associated with:</p> <ul style="list-style-type: none"> <li>Sideroblastic anemia</li> </ul>
Rouleaux (Fig. 2-15)	<p>Red blood cell aggregation that resembles a stack of coins. Cell clumping is due to an interaction with paraprotein and loss of Zeta potential.</p> <p>Associated with:</p> <ul style="list-style-type: none"> <li>Increased paraprotein or globulin</li> <li>Artifact</li> </ul>
Schistocyte (Fig. 2-16)	<p>Fragmented RBC, due to mechanical destruction by fibrin strands or prosthetic heart valves</p> <p>Associated with:</p> <ul style="list-style-type: none"> <li>DIC</li> <li>TTP</li> <li>Severe burns</li> <li>Hemolytic uremic syndrome (HUS)</li> <li>Renal graft rejection</li> <li>Prosthetic heart valve</li> </ul>
Sickle cell (Fig. 2-17)	<p>Sickle-shaped RBC</p> <p>Associated with:</p> <ul style="list-style-type: none"> <li>Homozygous hemoglobin S</li> </ul>
Spherocyte (Fig. 2-18)	<p>Dense, spherical RBC without central pallor, smaller than normal (usually seen with a dimorphic population)</p> <p>Due to abnormal cytoskeletal protein</p> <p>Associated with:</p> <ul style="list-style-type: none"> <li>Hereditary spherocytosis</li> <li>Immuno-hemolytic anemia</li> <li>Severe burns</li> <li>RBC transfusion</li> </ul>
Stomatocyte (Fig. 2-19)	<p>Mouth or cup like due to membrane defect with abnormal cation permeability</p> <p>Associated with:</p> <ul style="list-style-type: none"> <li>Hereditary stomatocytosis</li> <li>Alcoholism</li> <li>Liver disease</li> <li>Rh null phenotype</li> <li>A wide variety of medications and diagnoses, including malignant neoplasms, and cardiovascular disease</li> </ul>

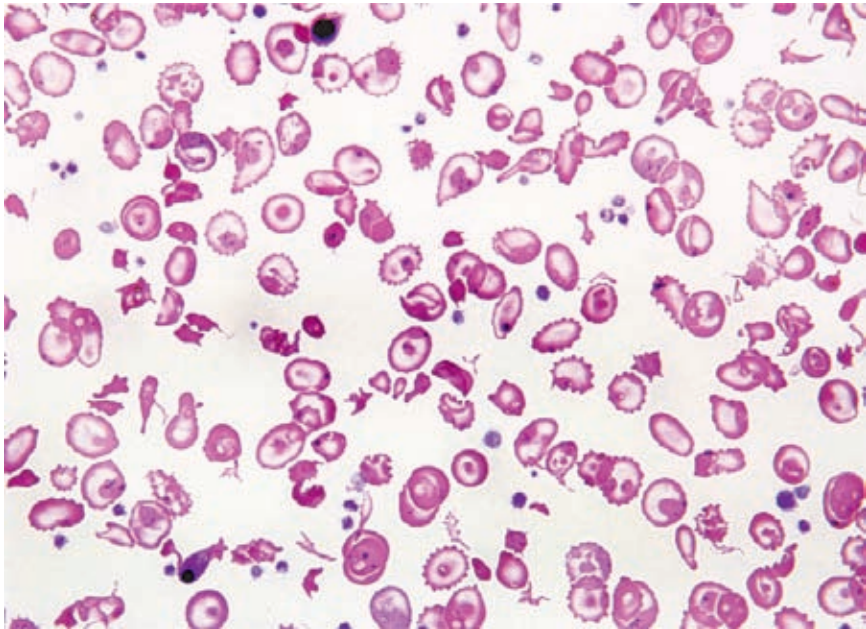
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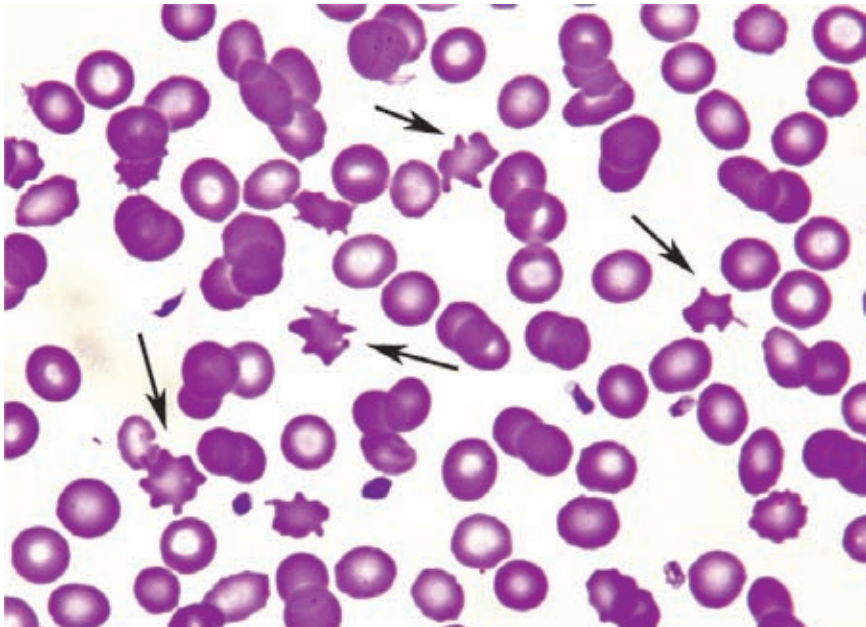
Target cell (Fig. 2-20)	Target-like appearance, due to increased surface membrane to volume ratio.  Associated with: Hemoglobin C disease Thalassemia Obstructive liver disease Post-splenectomy Iron deficiency
Teardrop cell (dacryocyte) (Fig. 2-21)	Teardrop shaped, due to mechanic distortion  Associated with: Myelofibrosis Myelophthitic anemia Thalassemias



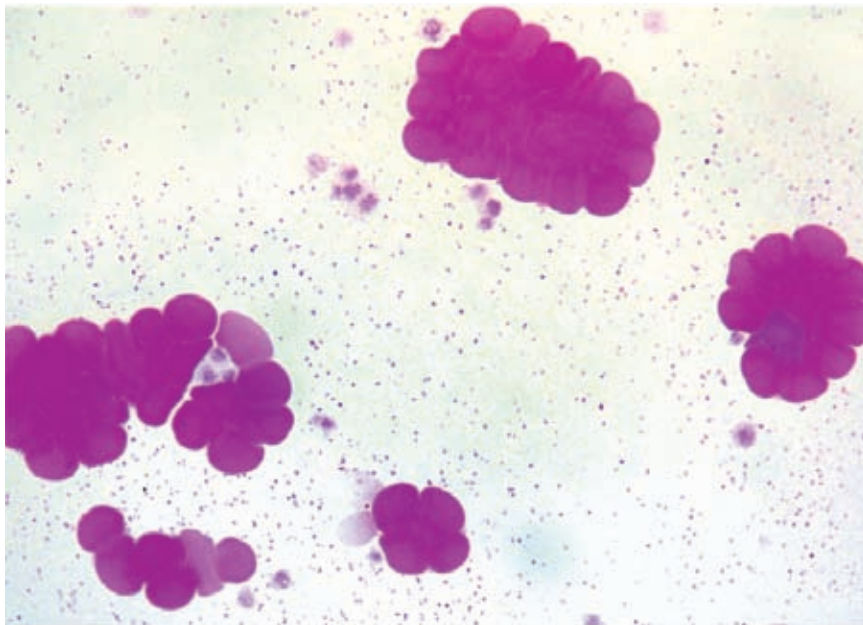
**Fig. 2-1: Anisocytosis** with erythrocytes of unequal size (Peripheral blood smear of megaloblastic anemia).



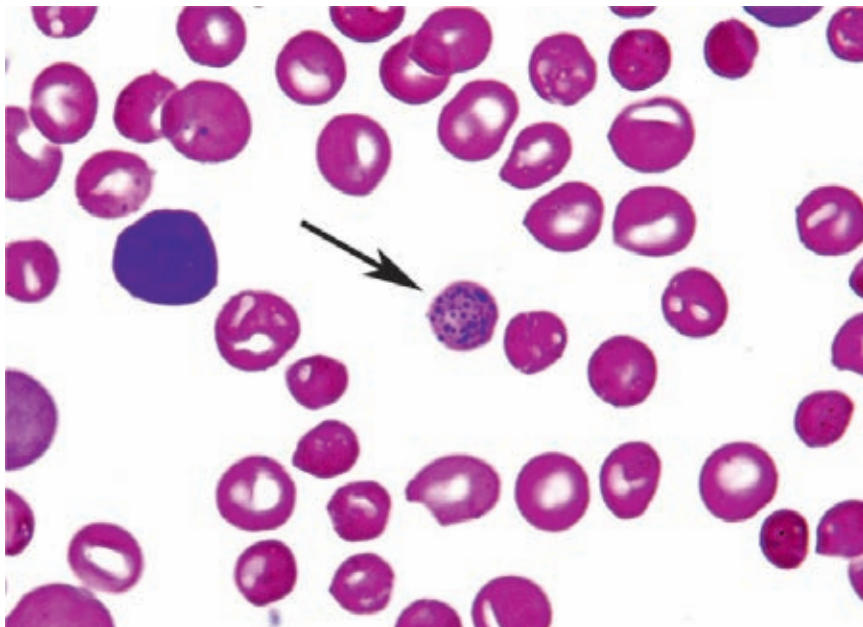
**Fig. 2-2: Poikilocytosis** showing abnormally shaped red blood cells (Peripheral blood smear of thalassemia major).



**Fig. 2-3: Acanthocytes** (arrows) showing irregular spiculated projections of cytoplasm (Peripheral blood smear).

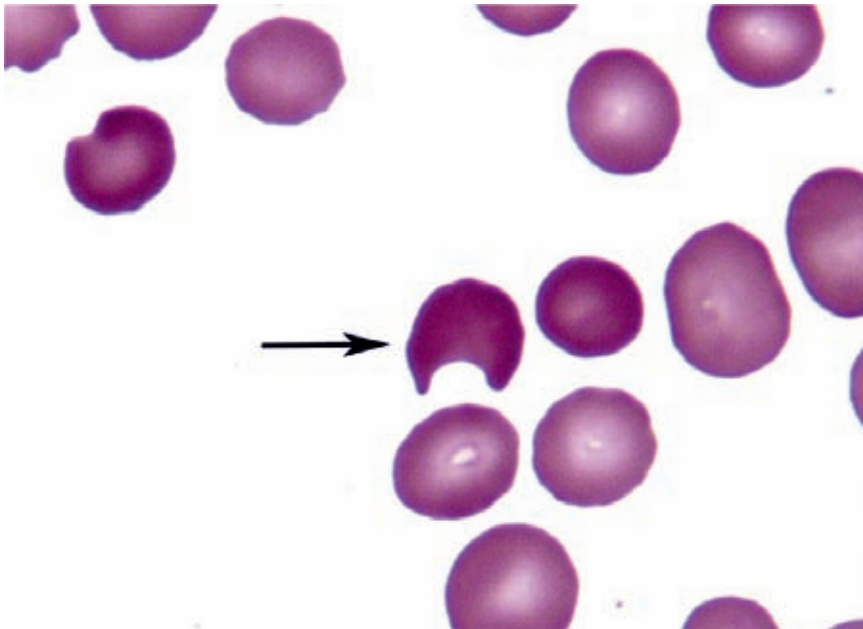


**Fig. 2-4: Autoagglutination** is clumping of red blood cells, the outline of individual cells may not be seen (Peripheral blood smear).

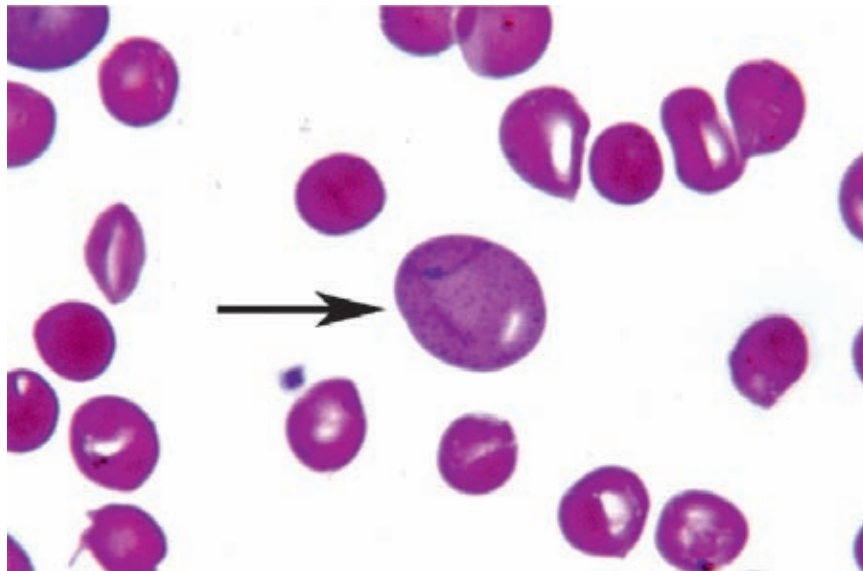


**Fig. 2-5: Basophilic stippling** (arrow) with punctate basophilic inclusions due to precipitated RNA (Peripheral blood smear).

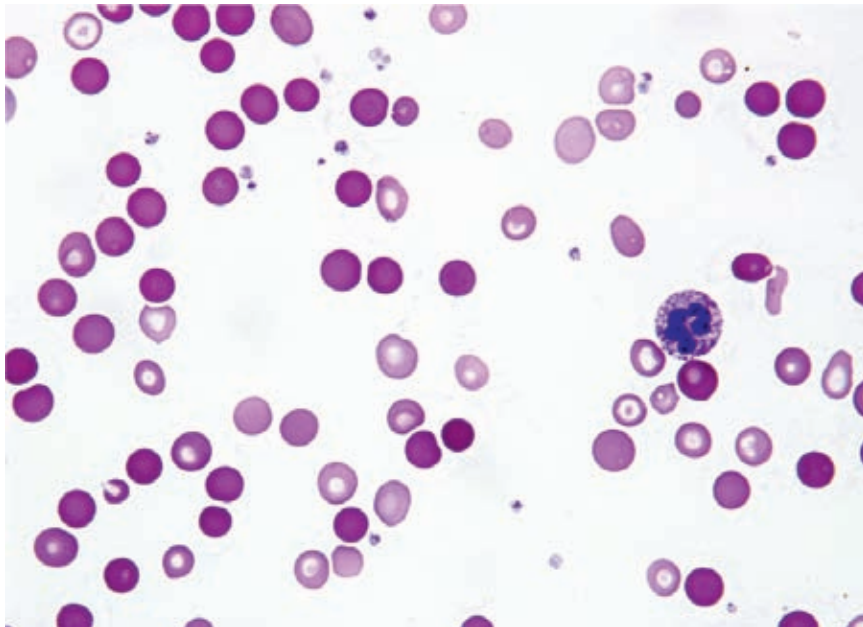




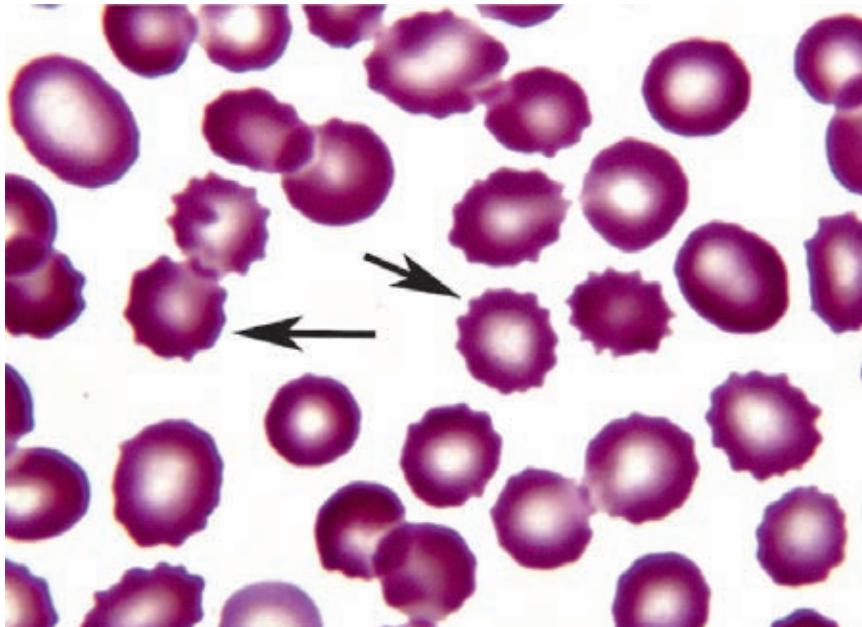
**Fig. 2-6: Bite cell** is a smooth semicircular (arrow) defect of red blood cell (Heinz body pitting by spleen) (Peripheral blood smear).



**Fig. 2-7: Cabot's ring** (arrow). Red-purple staining thread-like filaments in the shape of a "ring" or a "figure 8" in stained erythrocytes, often in association with basophilic stippling. Cabot's ring may also appear as granules in a linear array rather than as complete rings. Cabot rings are thought to be microtubules from a mitotic spindle or remnants of the nuclear membrane (Peripheral blood smear).

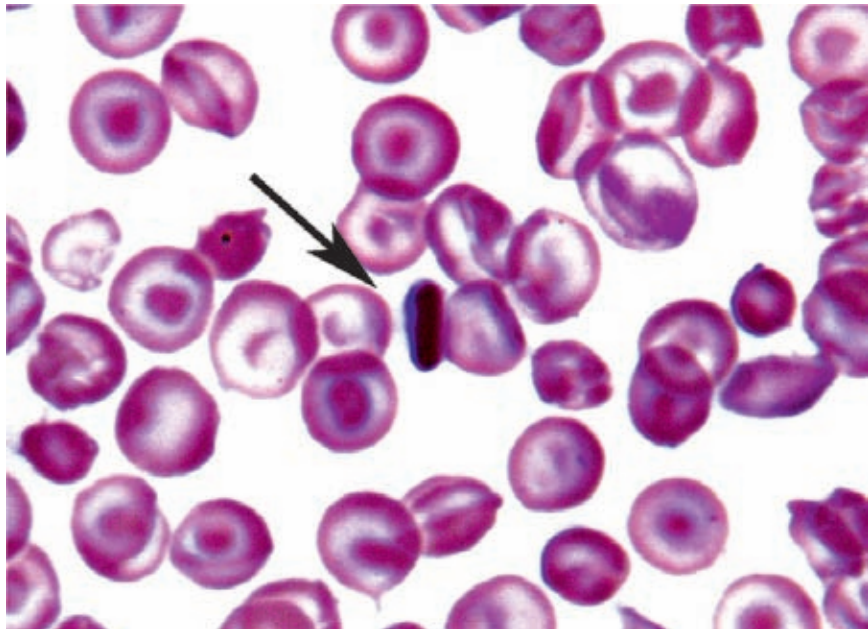


**Fig. 2-8: Dimorphic erythrocyte population.** Two distinctive erythrocyte populations are present. Associated with transfusion, MDS, Vitamin B<sub>12</sub>, folate or iron deficiencies (Peripheral blood smear).

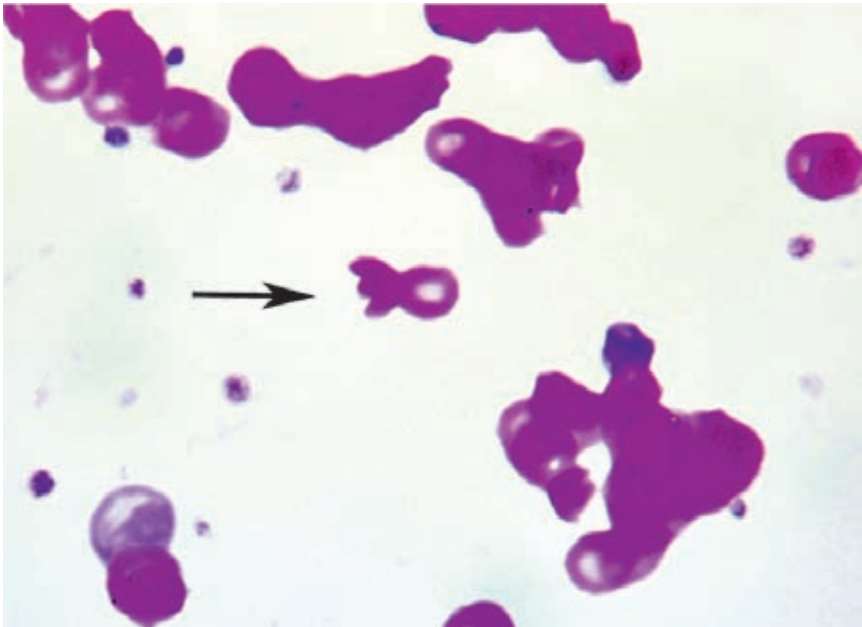


**Fig. 2-9: Echinocytes (burr cells)** showing evenly spaced spicules (arrows). Short evenly spaced spicules with preserved central pallor due to altered cell membrane lipid. Associated with renal disease (uremia), pyruvate kinase deficiency (PKD), microangiopathic hemolytic anemia, and artifact (Peripheral blood smear).

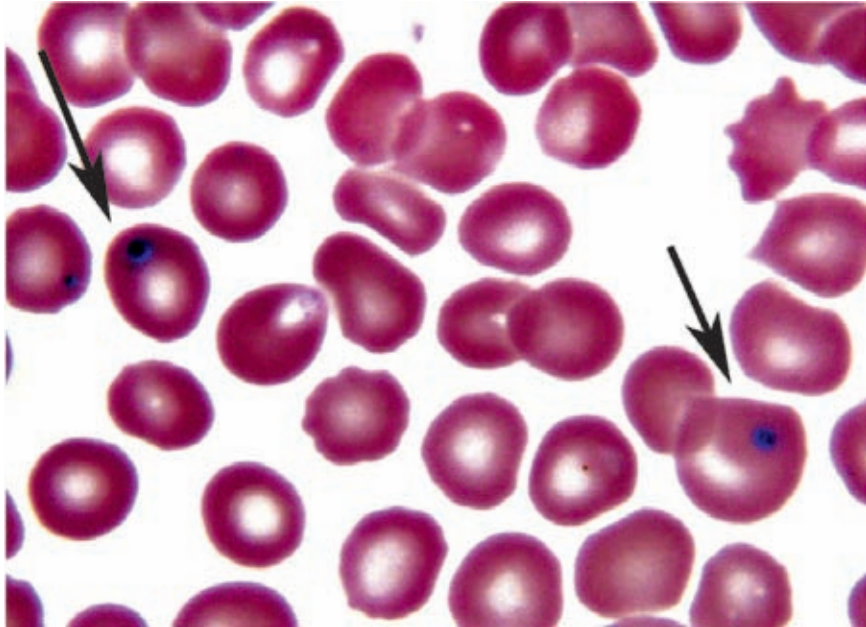




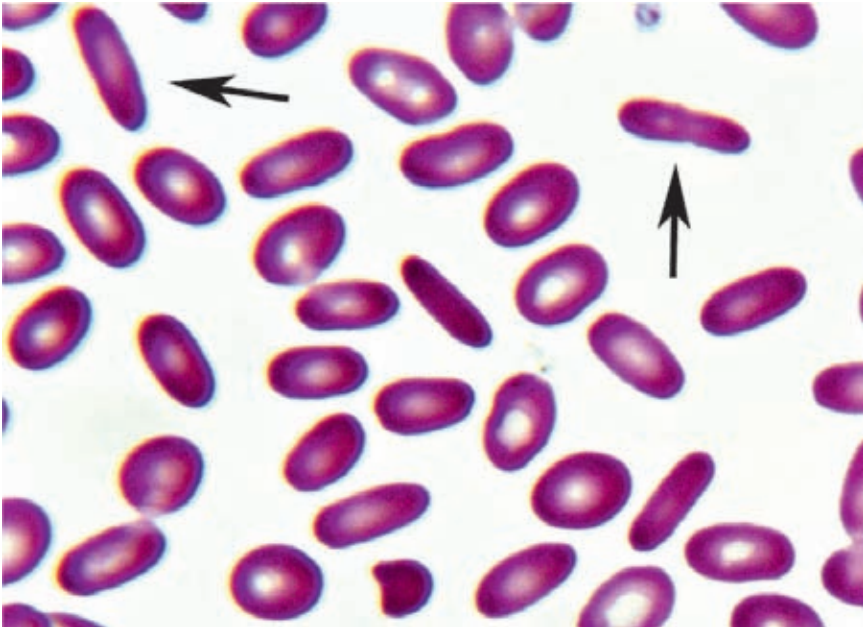
**Fig. 2-10: Hemoglobin C crystal** (arrow). Dark red, hexagonal shaped crystal associated with homozygous hemoglobin C disease (Peripheral blood smear).



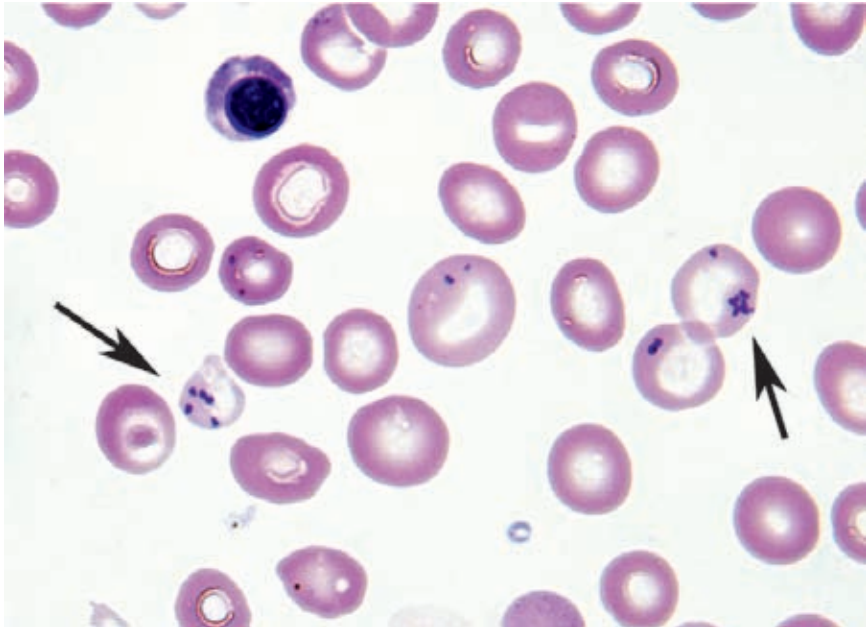
**Fig. 2-11: Hemoglobin SC** (arrow). Dark red deformed red blood cells with 1-2 finger-like projections may look like a mitten. Target cells are usually present (Peripheral blood smear).



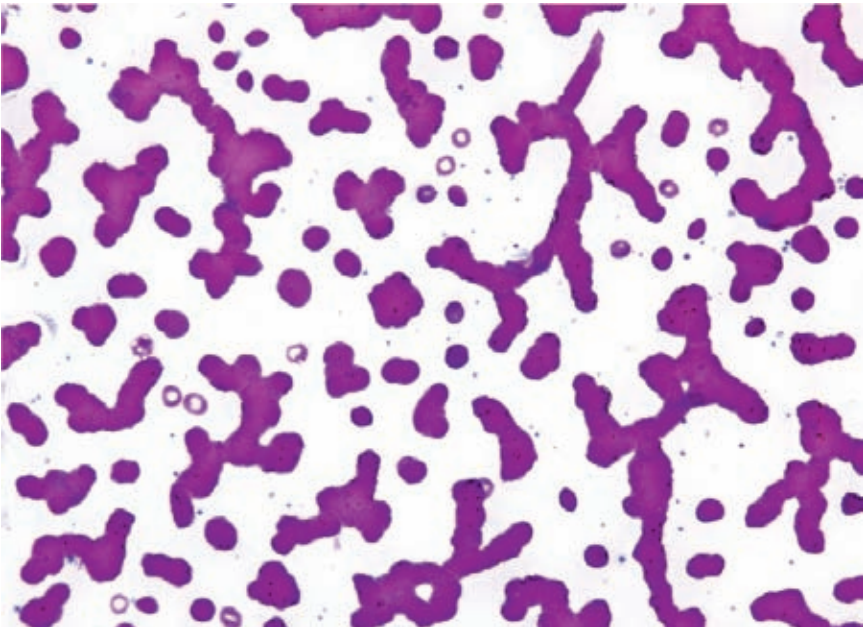
**Fig. 2-12: Howell-Jolly bodies** (arrows). Small basophilic cytoplasmic inclusions usually DNA nuclear remnants. Associated with postsplenectomy, hemolytic disease, and megaloblastic anemia (Peripheral blood smear).



**Fig. 2-13: Elliptocytes** showing elongated shapes (arrows). Elliptical shape due to abnormal cytoskeletal protein (Peripheral blood smear).

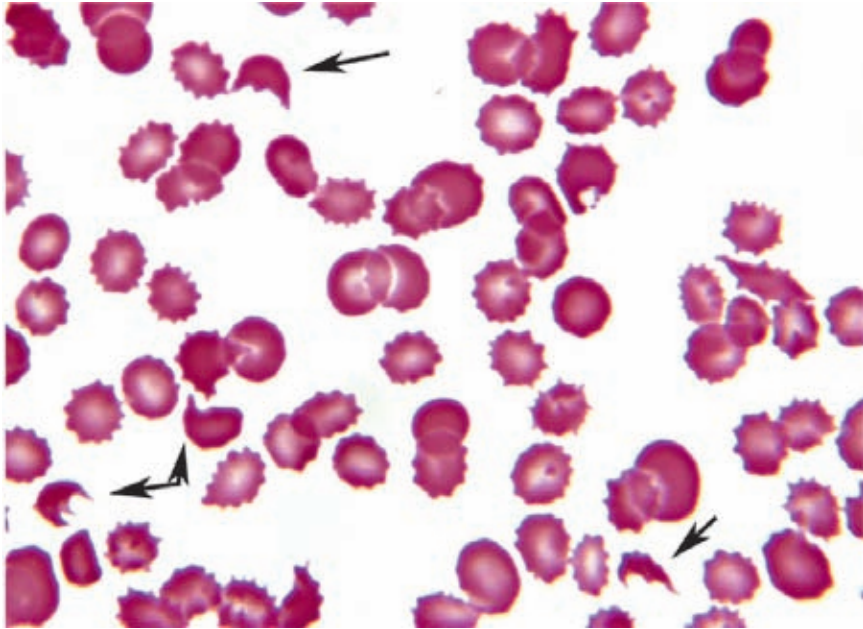


**Fig. 2-14: Pappenheimer bodies** (arrows) seen as small dense basophilic granules of iron-containing mitochondrial remnants or siderosome. Associated with sideroblastic anemia and postsplenectomy (Peripheral blood smear).

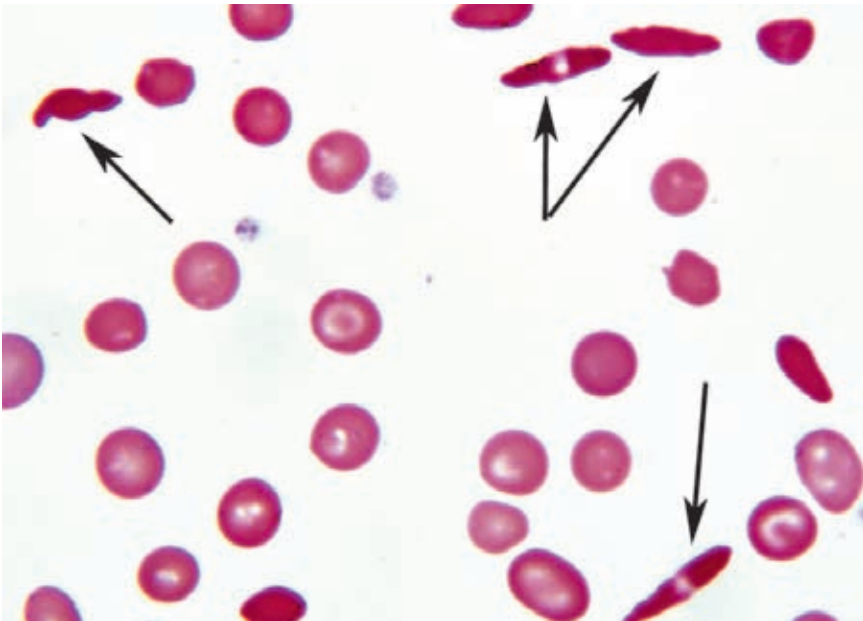


**Fig. 2-15: Rouleaux** seen as an aggregation of erythrocytes that resembles a stack of coins. Cell clumping is due to interaction with paraprotein and lost Zeta potential. Associated with increased paraprotein or globulin, or artifact (Peripheral blood smear).

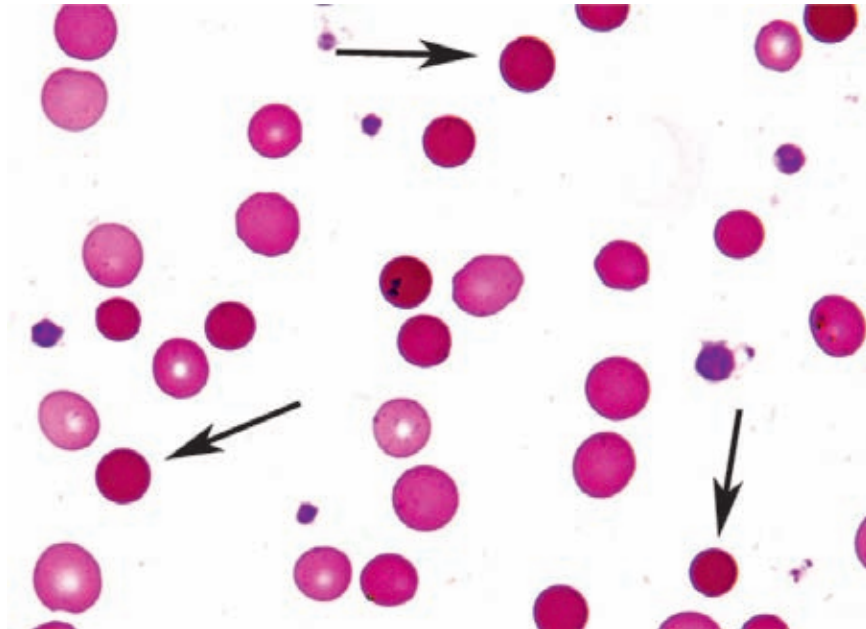




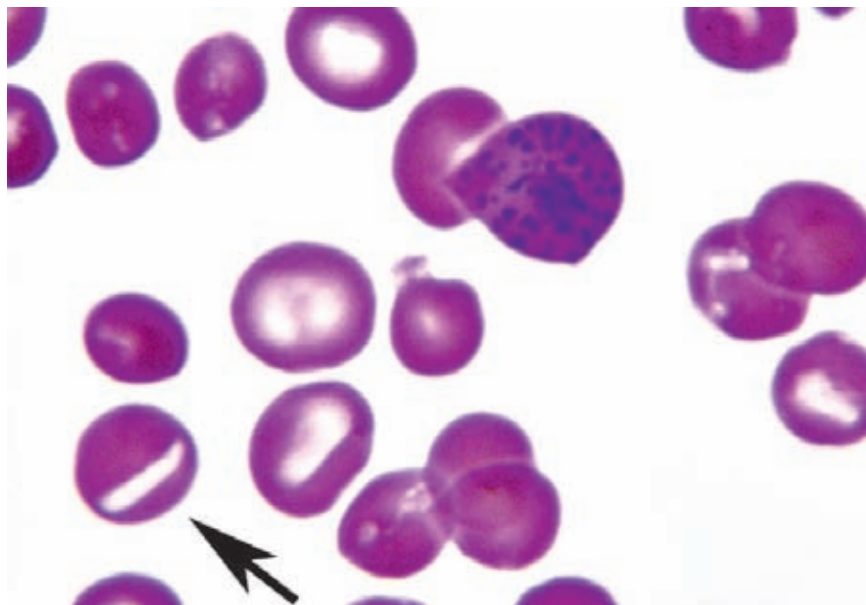
**Fig. 2-16: Schistocytes** seen as fragments of red blood cells (arrows) due to mechanic destruction by fibrin strands or prosthetic heart valve. Associated with DIC, TTP, severe burn, hemolytic uremic syndrome, renal graft rejection and prosthetic heart valves (Peripheral blood smear).



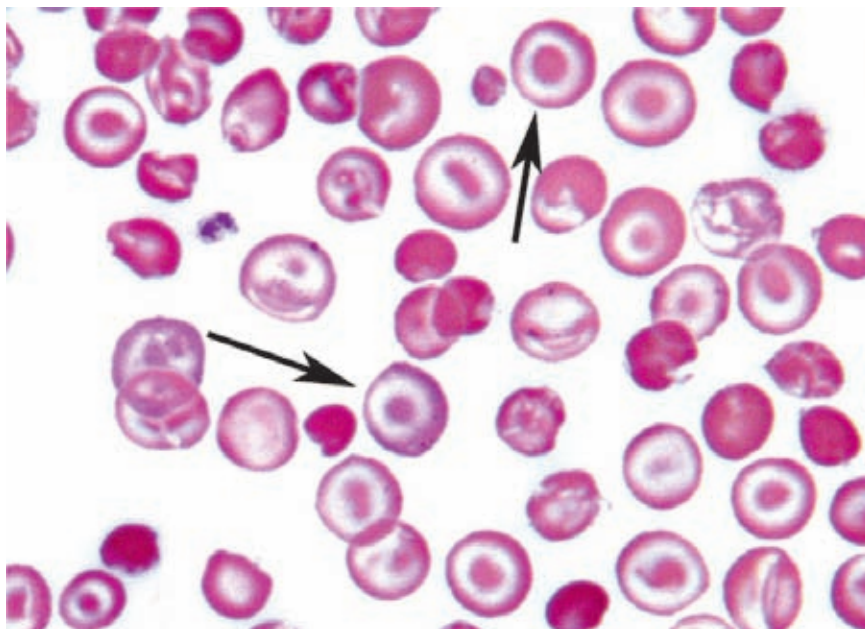
**Fig. 2-17: Sickle cells** seen as needle-like shapes (arrows). Due to deoxygenated, polymerized HbS (Peripheral blood smear).



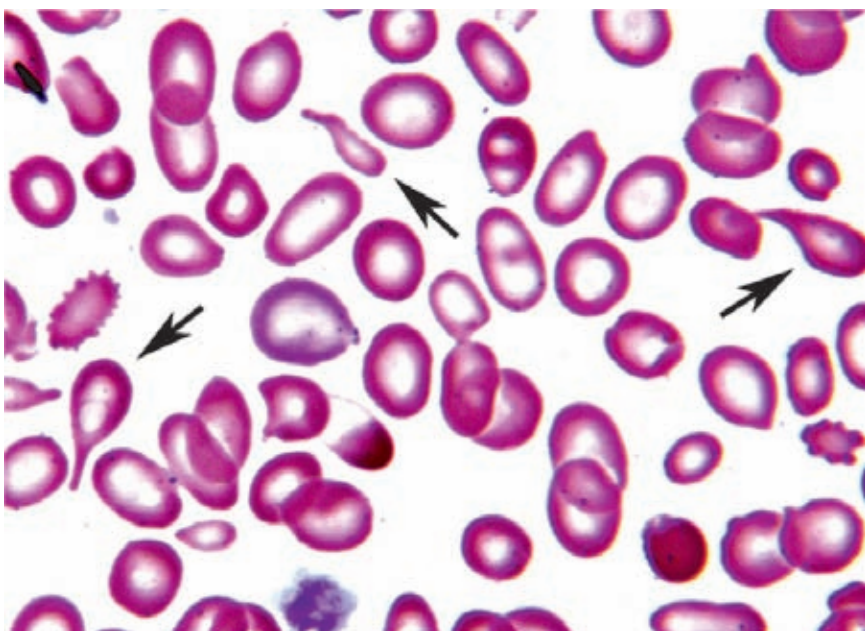
**Fig. 2-18: Spherocytes** seen as small, dense, spherical red blood cells (arrows) without central pallor due to an abnormal cytoskeleton protein. Associated with hereditary spherocytosis, immunohemolytic anemia, severe burns, transfused cells (Peripheral blood smear).



**Fig. 2-19: Target cells** showing a target-like appearance (arrow) due to erythrocytes with an elongated (mouth-like) area of central pallor. Associated with hereditary stomatocytosis, alcoholism, liver disease, Rh null phenotype, artifact and wide variety of medications and diagnoses (Peripheral blood smear).



**Fig. 2-20: Target cell** (arrows). Target-like or bull's-eye due to increase surface membrane to volume ratio. Associated with hemoglobin C, thalassemia, obstructive liver disease, postsplenectomy or iron deficiency (Peripheral blood smear).



**Fig. 2-21: Teardrop cells** (dacryocytes) showing teardrop-shaped red blood cells (arrows) due to mechanical distortion. Associated with myelofibrosis, myelophthisic anemia and thalassemias (Peripheral blood smear).

## *Anemia*

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Anemias can be classified according to red blood cell size (mean cell volume, MCV) or other pathophysiological changes such as anemia related to diminished production or accelerated loss of red blood cells, hemoglobinopathy, etc. Microcytic anemia  $MCV < 80$  fL is due to either iron deficiency or thalassemia. Macrocytic anemia ( $MCV > 100$  fL) may be due to megaloblastic causes (folate or vitamin B<sub>12</sub> deficiency) or nonmegaloblastic causes (myelodysplasia or antiretroviral drugs). Severely macrocytic anemia ( $MCV > 125$  fL) is usually due to either megaloblastic anemia or myelodysplasia. The differential diagnosis of microcytic anemia, normocytic anemia and macrocytic anemia are as follows:

### **Microcytic Anemia ( $MCV < 80$ fL)**

1. Iron deficiency anemia
2. Thalassemia
3. Sideroblastic anemia
4. Anemia of chronic disease

### **Normocytic Anemia ( $MCV$ 80-100 fL)**

#### **a. Low reticulocyte count**

1. Iron deficiency (early)
2. Anemia of chronic disease
3. Chronic renal disease (low erythropoietin)
4. Anemia of endocrine disorders (hypothyroidism, adrenal insufficiency, or hypopituitarism)
5. Primary bone marrow disorder
  - Aplastic anemia
  - Malignancy
  - Myelofibrosis
  - MDS

#### **b. High reticulocyte count**

1. Blood loss
2. Hemolysis

#### **c. Macrocytic anemia ( $MCV > 100$ fL)**

1. Megaloblastic anemia (folic acid or vitamin B<sub>12</sub> deficiency)
2. Liver disease (target cells)
3. Alcoholism
4. Reticulocytosis

5. Hypothyroidism
6. Myelodysplastic syndrome
7. Antiretrovirals (e.g. AZT) or chemotherapy.

### *Hypochromic/Microcytic Anemia*

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Hypochromic/microcytic anemia is characterized by a MCV of <80 fL and often hypochromatic. These anemias can be divided into three groups:

1. Disorders of iron metabolism or utilization (iron deficiency and anemia of chronic disease).
2. Disorders of globulin protein synthesis (Thalassemia)
3. Disorders of heme synthesis (Sideroblastic anemia).

### **Iron Deficiency**

Iron deficiency is the most common cause of anemia worldwide. The causes include deficient diet, decreased absorption, increased requirements (pregnancy or lactation), blood loss (GI tract, menstruation, and blood donation), hemoglobinuria, and iron sequestration (pulmonary hemosiderosis). Aside from circulating red blood cells, the major iron stores in the body are ferritin and hemosiderin in macrophages.

1. **Clinical presentation:** Easy fatigability, tachycardia, palpitations and tachypnea on exertion, smooth tongue, brittle nails, and cheilosis. Dysphagia may be related to the formation of esophageal webs (Plummer–Vinson syndrome). Many iron-deficient patients develop pica, craving for specific foods (ice chips, etc.) which are often not rich in iron.
2. **Laboratory findings:** Depletion of iron storage (bone marrow biopsy), low serum ferritin (< 12 mcg/L is a highly reliable indicator of iron deficiency. Serum iron values decline to < 30 mcg/dL and transferrin levels rise, leading to transferrin saturation of < 15%.
3. **Differential diagnosis:** Other causes of microcytic anemia include anemia of chronic disease, thalassemia, and sideroblastic anemia (Table 2-2 and Figs 2-22A and B).

### **Sideroblastic Anemias**

Sideroblastic anemias are a group of disorders characterized by ineffective erythropoiesis. The key finding is a **dimorphic population** of red blood cells with a high RDW, presence of ringed sideroblasts in the bone marrow,



**TABLE  
2-2****Iron studies in hypochromic anemias**

	Serum iron	TIBC	% saturation	SSTR	BM iron
Iron deficiency	↓	↑	↓ (<10%)	↑	↓
Thalassemia	↑ or normal	↓ or normal	↑ or normal	variable	↑
Sideroblastic anemia I	↑	↓ or normal	↑	variable	↑
Chronic disease	↓	↓	↓ (>10%)	normal	↑

TIBC: total iron binding capacity

% saturation: percentage transferrin saturation

SSTR: soluble serum transferrin receptor

elevated serum iron levels, and elevated transferrin saturation. Sideroblastic anemia can be inherited or acquired (MDS, alcohol, lead, cycloserine, pyrazinamide, chloramphenicol, and isoniazid). The common finding is an abnormal deposition of iron within the mitochondria of erythroblasts.

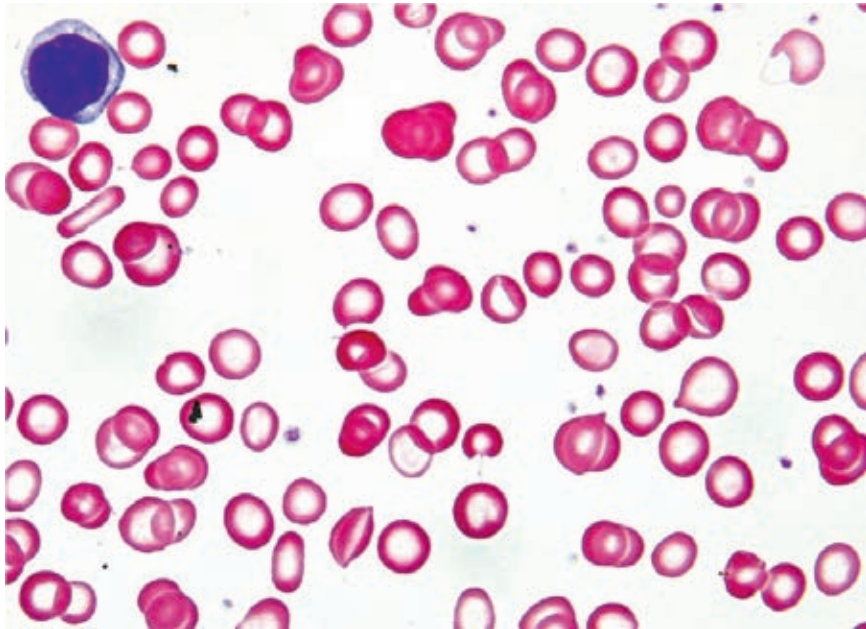
The inherited type usually occurs in males (X-linked) and is rare in females. Some of these patients have mutations in the gene (ALA-S) located on the X-chromosome which codes for erythroid specific enzyme (Figs 2-23A and B).

### Anemia of Chronic Disease

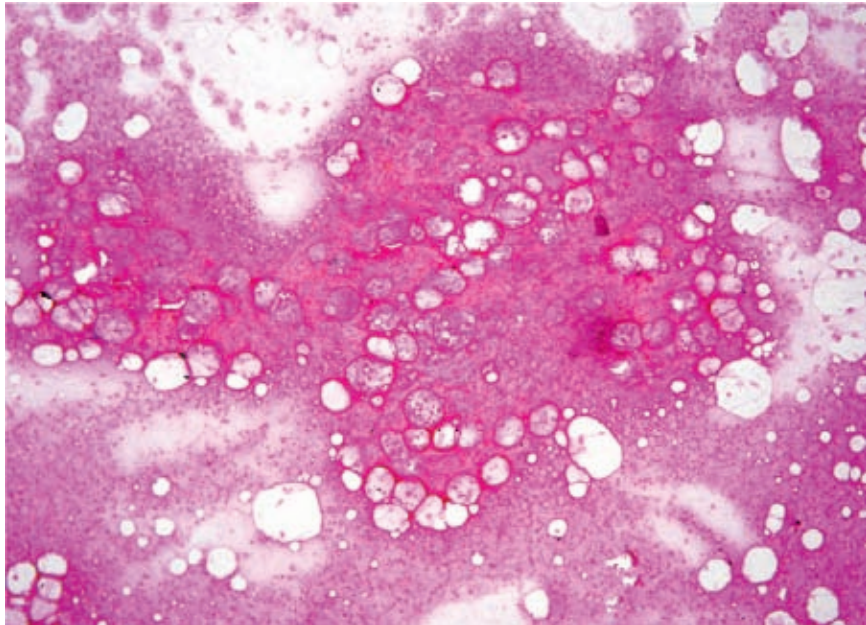
Anemia of chronic disease is associated with multiple iron related abnormalities. Iron studies show decreased serum iron, and decreased total iron binding capacity (reflecting transferrin concentration) and normal to increased iron (ferritin) storage. Anemia of chronic disease is related to **inhibitory cytokines** (TNF- $\beta$ , IL-1, IL-6, and IFN- $\gamma$ ) and **Hepcidin** (a hepatic peptide hormone produced by liver). Anemia of chronic disease is usually normocytic, but may be hypochromic, microcytic and normochromic.

### Anemia Associated with AIDS

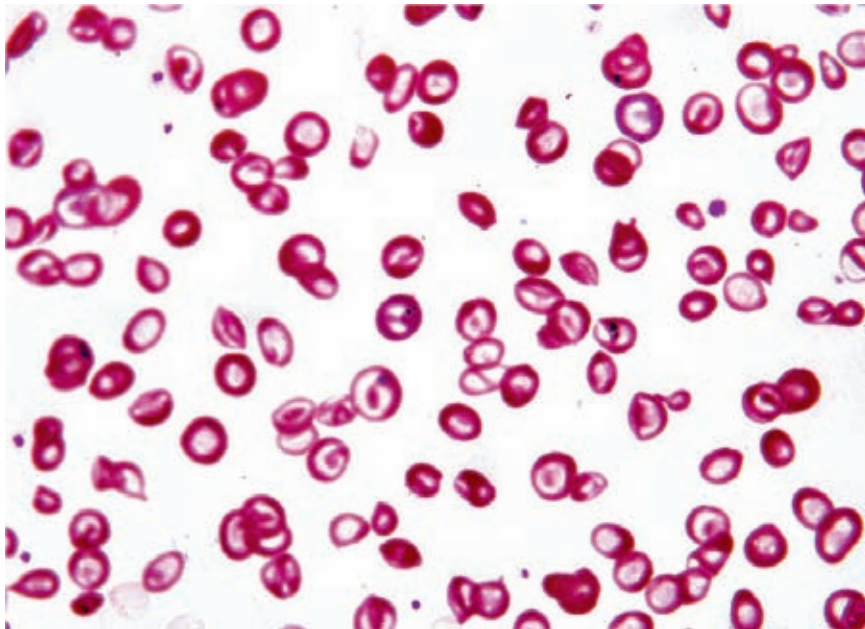
AIDS-related anemia is multifactorial. The HIV infection itself, as well as anti-viral chemotherapy, may reduce the production of hematopoietic cell lineages. In addition, inflammatory cytokines may generate negative effects on erythropoietin production. The bone marrow is usually hypocellular and undergoes **serous atrophy**. Serous atrophy is a phenomenon that amorphous gelatinous material replacing bone marrow fat and hematopoietic cells (Figs 2-24A and B).



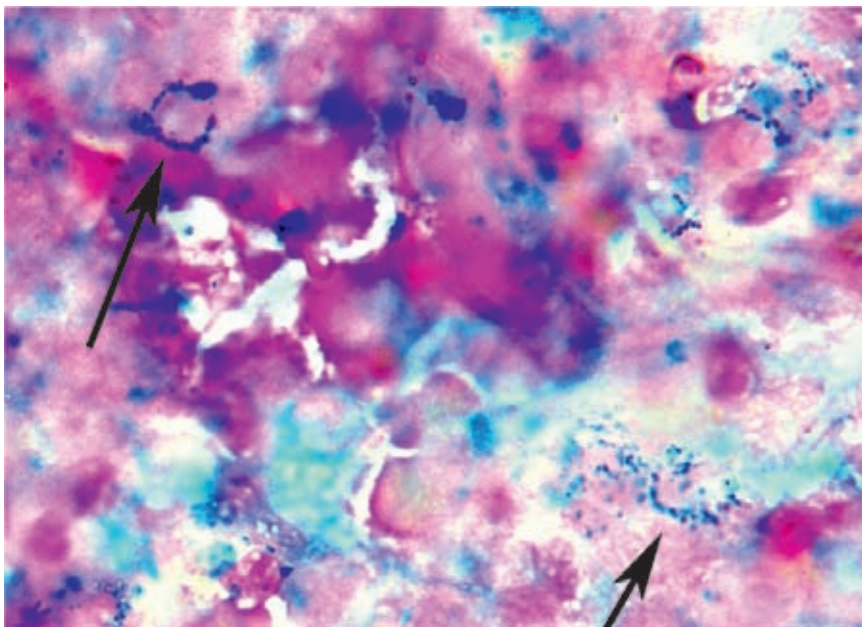
**Fig. 2-22A: Iron deficiency anemia** showing variably-sized erythrocytes. Most cells are smaller than the nucleus of the small lymphocyte (this comparison can be used as an approximate measure of microcytosis) (Peripheral blood smear).



**Fig. 2-22B: Iron deficiency anemia.** Iron stain (Perl's) shows absence of stainable iron in bone marrow spicules (Bone marrow aspirate).

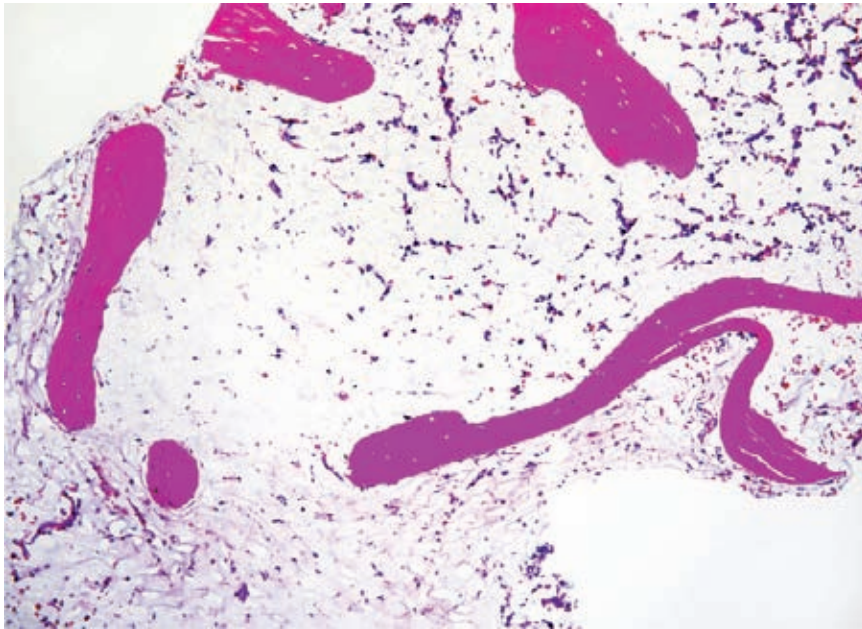


**Fig. 2-23A: Sideroblastic anemia** showing a dimorphic population of microcytic and normocytic red blood cells (Peripheral blood smear).

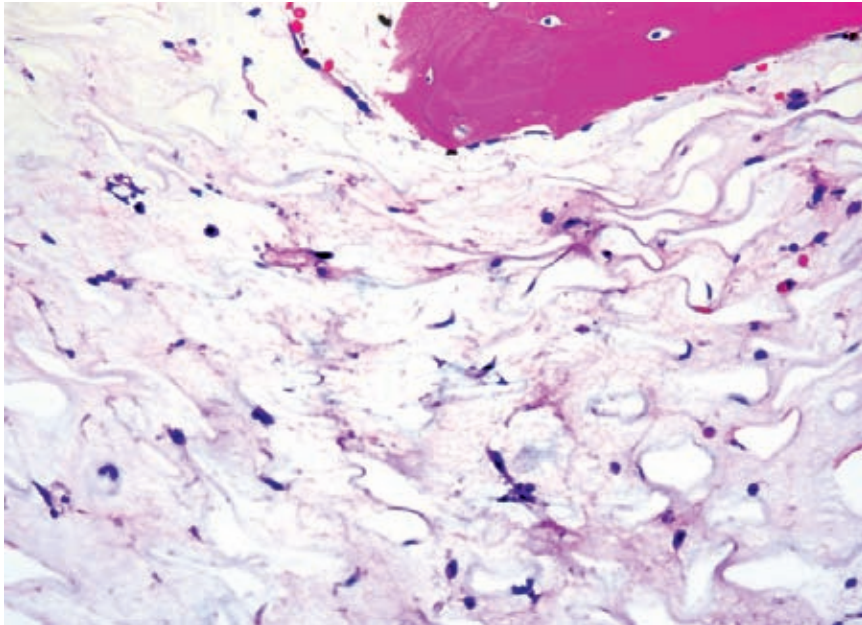


**Fig. 2-23B: Sideroblastic anemia.** Iron stain (Perl's) showing erythroblasts with partial or complete rings of iron granules around their nuclei (arrows).





**Fig. 2-24A: Serous atrophy.** Amorphous gelatinous material replaces bone marrow fat and hematopoietic cells (low power). This is a bone marrow biopsy from a patient with HIV infection (Bone marrow section).



**Fig. 2-24B: Serous atrophy (high power)** (Bone marrow section).

### Anemia Associated with Chronic Renal Failure

Chronic renal failure related anemia is multifactorial, including:

1. Hypoproliferation of erythroid lineage ( decreased erythropoietin production)
2. Chronic hemolysis (decreased RBC survival)
3. Iron or folate deficiency
4. Toxic effect of dialysis.

### Anemia Associated with Lead Poisoning

Lead inhibits two important enzymes in heme synthesis,  $\delta$ -aminolevulinic acid (ALA) dehydratase and ferrochelatase. Lead also inhibits pyrimidine 5'-nucleotidase which is one of the enzymes responsible for RNA degradation. The histological finding of basophilic stippling ( see Fig. 2-5) of red blood cells is due to precipitation of undegraded RNA.

### *Megaloblastic Anemias*

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The megaloblastic anemias are a group of disorders characterized by ineffective erythropoiesis and distinctive morphology in the bone marrow.

Peripheral blood usually shows oval macrocytes with considerable anisocytosis and poikilocytosis, MCV is usually  $>100$  fL, unless a cause of microcytosis is present. Some of the neutrophils are hypersegmented (more than five nuclear lobes). **Mild leukopenia and thrombocytopenia may be present.**

In severe megaloblastic anemia, bone marrow is hypercellular with an accumulation of immature cells. The megaloblastic changes include immature erythroblast nucleus despite maturation of the cytoplasm (nuclear-cytoplasmic asynchrony), eccentric lobulated nuclei or nuclear fragments. Giant and abnormally shaped metamyelocytes and enlarged megakaryocytes are present.

### Causes of Megaloblastic Anemia

1. Vitamin B<sub>12</sub> deficiency (Addison's disease, hypoparathyroidism, atrophic gastritis).
2. Folate deficiency (celiac disease, tropical sprue, alcoholism, pregnancy).
3. Chemotherapy (folic acid antagonists, such as methotrexate).

Folate is used for biosynthesis of purines, thymidine, and methionine by transport of one-carbon fragments. Vitamin B<sub>12</sub> (cobalamin) is required for the conversion of methyl-malonyl coenzyme A (CoA) to succinyl CoA, and the conversion of homocysteine to methionine. Folate and Vitamin B<sub>12</sub> (cobalamin) deficiency affects DNA synthesis but not RNA synthesis. Normal storage of Vitamin B<sub>12</sub> usually last 2-5 years while a folic acid stores only last 3-5 months.

The absorption of cobalamin occurs in the small intestine. **Intrinsic factor** is synthesized and secreted by the parietal cells of the stomach to form an intrinsic factor—cobalamin complex. This complex is absorbed in the terminal ileum, and Vitamin B<sub>12</sub> is then released into the portal blood. In the portal blood, cobalamin is complexed with **transcobalamin** (TC, previously known as **transcobalamin II**), 10-30% of Vitamin B<sub>12</sub> binds to TC to be delivered to liver, bone marrow and other sites. TC is synthesized by many types of cells, including enterocytes, hepatocytes, endothelial cells, mononuclear phagocytes, fibroblasts, and hematopoietic precursors in the marrow.

The plasma **haptocorrin** (HC, previously known as TCI, TCII and R proteins) carries most (70–90%) of the circulating cobalamin and delivers exclusive to the liver.

### Anemia Associated with Chronic Alcoholism

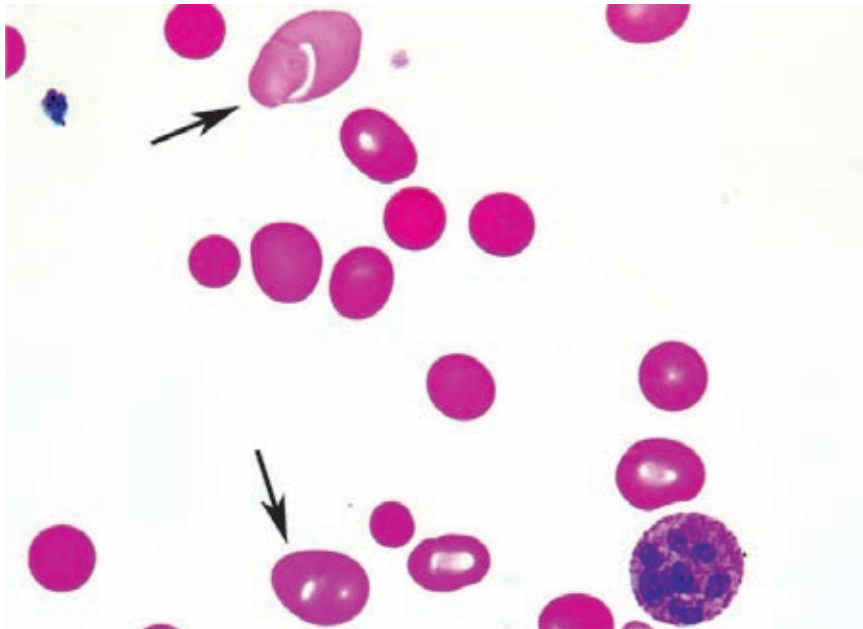
Alcohol has direct toxic effects on hematopoietic elements (decreased bone marrow cellularity and vacuolization of erythroid precursors), and can cause folate and Vitamin B<sub>12</sub> deficiencies (Figs 2-25A and B).

### *Aplastic Anemia and Hypoplastic Anemia (Table 2-3)*

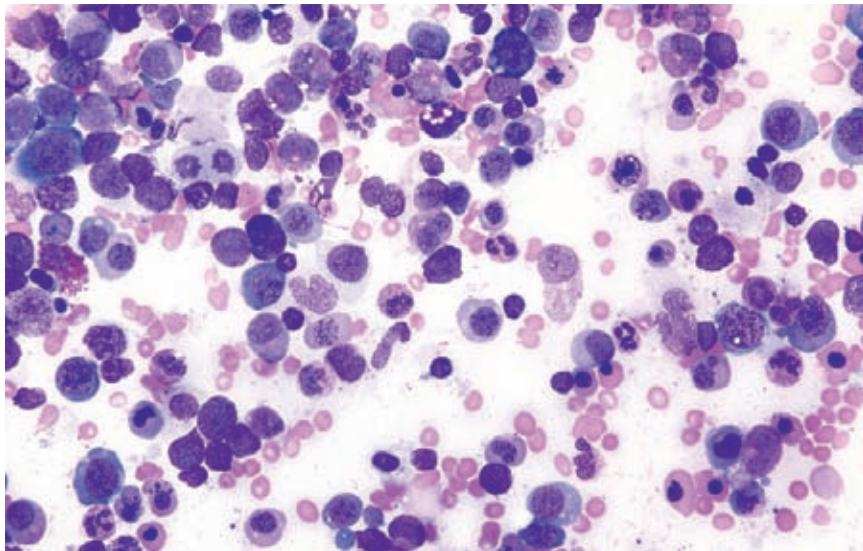
1. **Fanconi anemia or Fanconi syndrome** is an autosomal recessive disease with 65% of cases related to the FANCA gene mutation (located on chromosome 16q24.3). In rare cases, the gene mutation may be related to BRCA2. These patients are also predisposed to ovarian, breast and other cancers. Clinical features include growth retardation, short stature, skeletal anomalies, and café-au-lait spots. Blood counts and marrow cellularity are often normal until 5 to 10 years of age. In the late stage, the marrow becomes hypocellular (Fig. 2-26); the patients present with thrombocytopenia, leukopenia, macrocytic anemia and aplastic anemia.

The disease often progresses to MDS and AML.

Chromosomal typing of peripheral lymphocytes shows **spontaneous chromosomal breakage** (Fig. 2-26).

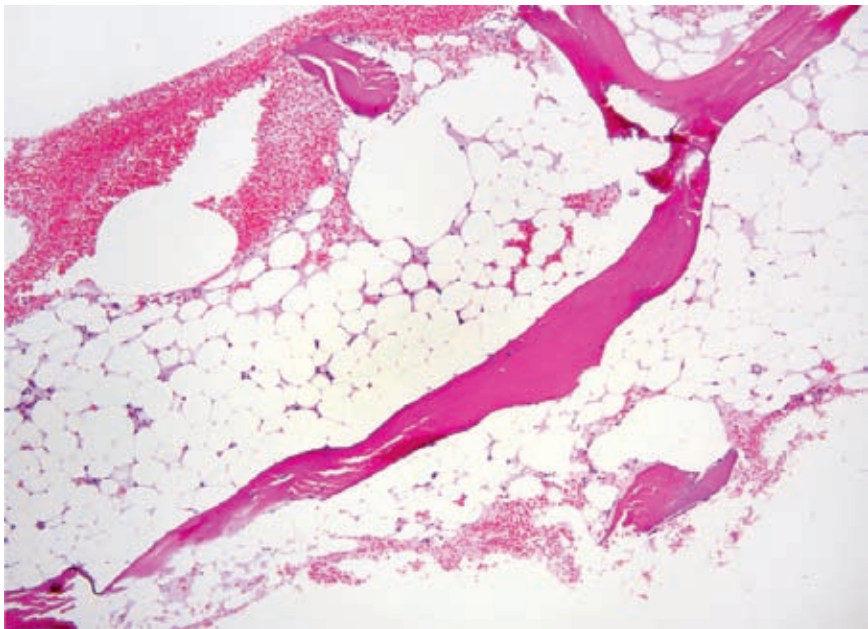


**Fig. 2-25A: Megaloblastic anemia.** The erythrocytes exhibit marked anisocytosis and poikilocytosis, ranging from oval macrocytes (arrows) to microcytes. A hypersegmented polymorphonuclear neutrophil is also present (Peripheral blood smear).



**Fig. 2-25B: Megaloblastic anemia.** Nuclear maturation is slowed whereas cytoplasmic maturation is relatively unimpeded. The impaired nuclear maturation is seen as open, loose, immature chromatin. This disparity between nucleus and cytoplasm is known as nuclear-cytoplasmic asynchrony. Giant bands and hypersegmented polymorphonuclear neutrophils are common (Bone marrow aspirate).





**Fig. 2-26: Aplastic anemia.** The bone marrow is markedly hypocellular, hematopoietic cells are markedly decreased in number (Bone marrow section).

2. **Dyskeratosis congenita** is a rare X-linked inherited aplastic anemia. The affected gene is located on Xq28. Mutations in the telomerase-related genes results in markedly shortened telomeres resulting in genomic instability, and cell apoptosis. Males are more common affected, however, females may also be affected as both autosomal dominant and recessive inheritance patterns may occur.
- The incidence of squamous cell carcinoma is increased in these patients.

TABLE 2-3	Aplastic anemia and hypoplastic anemia
Inherited aplastic anemia	Fanconi syndrome Dyskeratosis congenita Shwachman-Diamond syndrome
Acquired aplastic anemia	Idiopathic Drug Toxins Infections (viral) Paroxysmal nocturnal hemoglobinuria



3. **Shwachman-Diamond syndrome** is autosomal recessive disease due to mutations of the SBDS (Shwachman-Bodian-Diamond syndrome) gene on chromosome 7q11. This induces accelerated apoptosis via the FAS ligand pathway. Clinical features include exocrine pancreatic deficiency, neutropenia, anemia, thrombocytopenia, short stature, and mental retardation. Death from overwhelming sepsis is common. In late stage, patients may progress to MDS and AML (Table 2-3).

### *Pure Red Blood Cell Aplasia*

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These conditions are associated with normocytic anemia, marked reduction of reticulocytes (<1%) seen in the peripheral blood, and absent/rare erythroid precursors in the bone marrow.

Pure red blood cell aplasia is divided into three categories (Table 2-4):

1. Inherited
2. Acquired, transient erythroid hypoplasia
3. Acquired, sustained erythroid hypoplasia.

### **Inherited Pure Red Blood Cell Aplasia**

**Diamond-Blackfan anemia (congenital hypoplastic anemia)** is characterized by severe macrocytic anemia, reticulocytopenia in the peripheral blood and by decreased numbers of erythroid precursors in the bone marrow. Diamond-Blackfan anemia usually presents in infancy or early childhood. Short stature or other congenital anomalies are present in one-third of the patients.

### **Acquired Pure Red Blood Cell Aplasia**

Pure red blood cell aplasia occurs in about 5% of the patients with **thymoma** (accounts for approximately 10% of pure red blood cell aplasia). Hematopoietic neoplasms, especially **CLL** and **large granular lymphocytic leukemia** have been associated with pure red blood cell aplasia. Some autoimmune diseases, infections, and drugs are also associated with pure red blood cell aplasia, such as SLE, Sjögren disease, **parvovirus B19** viral infection, phenytoin, azathioprine, and isoniazid.

Aplastic anemias with an increased fetal hemoglobin level are present in Fanconi and Diamond-Blackfan syndrome, but not transient erythroblastopenia.

**TABLE  
2-4****Three categories of pure red blood cell aplasia (erythroid hypoplasia)**

Inherited	Diamond-Blackfan anemia
Acquired, transient	Transient erythroblastopenia of childhood Parvovirus infection (usually transient)
Acquired, sustained	Idiopathic Thymoma Large granular lymphocytic leukemia (T-cell) Chronic lymphocytic leukemia Clonal myeloid diseases (especially 5q syndrome) Viral infection (other than parvovirus) Drug treatment Collagen vascular diseases

### *Anemia Related to Hemolysis*

There are two types of hemolysis, intravascular hemolysis and extravascular hemolysis due to sequestration and destruction by the mononuclear phagocytic system.

**Laboratory evaluation of hemolysis:** Normally serum haptoglobin binds free hemoglobin; therefore, decreased level of haptoglobin is indicative of hemolysis. LDH1 (predominantly red blood cell and myocardium) is increased.

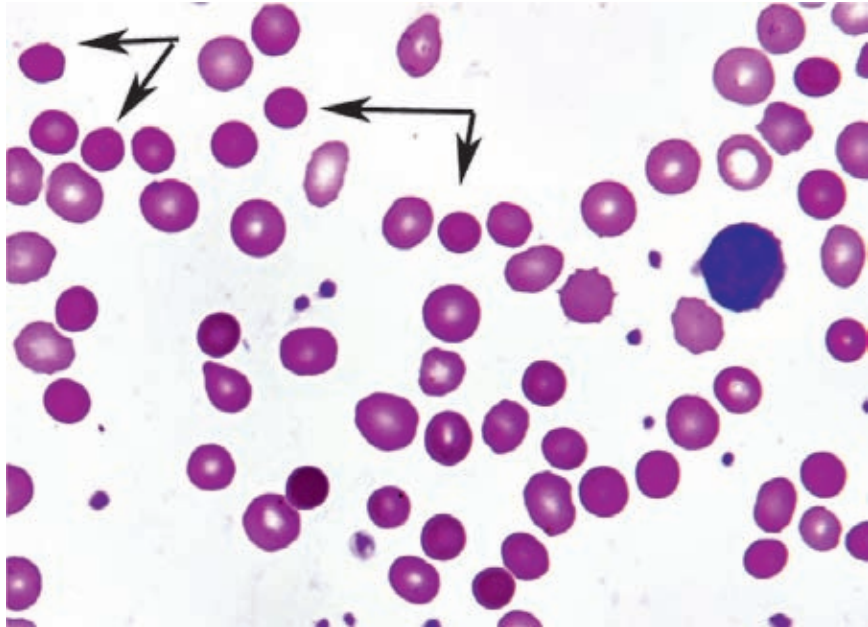
### **Hereditary Erythrocyte Membrane Defects**

The red blood cell cytoskeleton is formed by interactions of numerous proteins, including **spectrin, actin, ankyrin, protein 4.1, protein 4.2, and AE1 (band 3)**. Defects of these membrane cytoskeleton elements result in the alteration of red blood cells.

### ***Hereditary Spherocytosis***

**Hereditary spherocytosis (HS)** is a disorder involving the red blood cell membrane, leading to chronic hemolytic anemia of variable severity. The membrane defect is an abnormality in membrane cytoskeletal spectrin, ankyrin, AE1 (band 3), and protein 4.1 or 4.2. This defect leads to decreased surface-to-volume ratio that result in a spherical-shaped cell. These spherical cells are less deformable and unable to pass through fenestrations in the splenic red pulp. Hemolysis takes place because of splenic trapping of red blood cells, so that splenectomy is the treatment of choice. The anemia is of

variable severity, and the hematocrit may be normal. Reticulocytosis is always present. The peripheral blood smear shows the presence of small spherical cells that have lost their central pallor (Fig. 2-27), which is usually only a small percentage of the red blood cell population. Hereditary spherocytosis is associated with microcytosis and an increased mean corpuscular hemoglobin concentration (MCHC). The Coombs test is negative.



**Fig. 2-27: Spherocytosis** showing red blood cells that have lost their central pallor (arrows), usually accounting for only a small percentage of the total red blood cell population. Hereditary spherocytosis is associated with microcytosis and an increased mean corpuscular hemoglobin concentration (MCHC) (Peripheral blood smear).

Hereditary spherocytosis is either an autosomal dominant (common, > 65% cases) or autosomal recessive (most common in Asian, but rare in the European population) inherited pattern. It is often diagnosed during childhood, but milder cases may be discovered incidentally later in adult life. Depending on the ability of the bone marrow to compensate for shortened red blood cell survival, anemia may or may not be present. Severe anemia (aplastic crisis) may occur in case of folic acid deficiency, or viral infection such as parvovirus B19. Chronic hemolysis causes cholelithiasis, leading to cholecystitis.

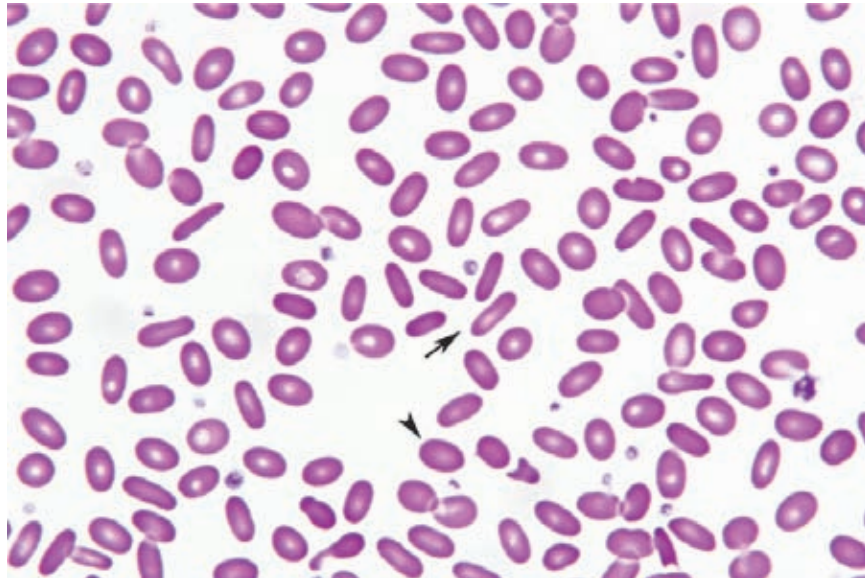
**Hereditary spherocytosis diagnostic laboratory tests:**

1. Osmotic fragility test, which shows increased fragility of red blood cells.
2. Flow cytometry for AE1 (band 3 protein), which shows decreased band 3 expression on the red blood cells.

***Hereditary Elliptocytosis***

**Hereditary elliptocytosis (HE)** has little or no effect on the shortening of red blood cell survival.

Hereditary elliptocytosis is characterized by the presence of elliptical erythrocytes (Fig. 2-28) on the peripheral blood smear. The principal defect in the erythrocyte is a mechanical weakness caused by abnormalities in the proteins involved with the membrane cytoskeleton, including  $\alpha$ -spectrin,  $\beta$ -spectrin, protein 4.1, and glycophorin C. The majority of patients are asymptomatic, and therapy is rarely necessary (Table 2-5).



**Fig. 2-28: Hereditary elliptocytosis** showing both elliptical-shaped (arrow), and oval-shaped (arrowhead) red blood cells (Peripheral blood smear).

### **Hereditary Erythrocyte Disorders due to Deficiencies of the Glycolytic Pathway**

Mature red blood cells do not have mitochondria; therefore, they derive their energy from glucose. This pathway is called **glycolytic, or Embden-Meyerhof pathway**.

**TABLE  
2-5****Inheritance patterns and cytoskeletal defect associated with HS and HE**

Hereditary spherocytosis	Autosomal dominant: Ankyrin $\beta$ -spectrin AE1 Protein 4.1	Autosomal recessive: $\alpha$ -spectrin (rare but severe) Protein 4.2
Hereditary elliptocytosis	Autosomal dominant $\alpha$ -spectrin (60%) Protein 4.1 (20-30%) $\beta$ -spectrin Glycophorin C	

Protein 4.1 deficiency is most common in North Africa

Protein 4.2 deficiency is most common in Japan, and rare in the European population.

Enzyme mutations associated with hemolytic anemia (rare, usually less than 100 cases reported) are hexokinase, glucose-6-phosphate isomerase, 6-phosphofructokinase, fructose biphosphate aldolase, phosphoglycerate kinase, and pyruvate kinase (Mnemonic: kinase, isomerase, and aldolase).

Enzyme mutations that do not cause hemolytic anemia are **lactate dehydrogenase, phosphoglycerate mutase, and enolase**.

- 1. Pyruvate kinase deficiency (PKD)** is most common enzyme deficiency of the glycolytic pathway, comprising about 90% of all cases. Fluorescent screening test (NADH-dependent conversion of pyruvate to lactate), red blood cell enzyme activity test, and DNA sequencing are used to screen and diagnose pyruvate kinase deficiency.
- 2. Glucose-6-phosphate dehydrogenase deficiency (G6PD) and drug-related extrinsic hemolytic anemia.** Approximately 10% of the red blood cell glucose is metabolized by the hexose monophosphate oxidative shunt. It uses glutathione to generate NADPH from NADP<sup>+</sup> in order to prevent cell oxidative injury.

G6PD has an X-linked inheritance pattern (only males are affected) Women who are carriers may have two populations of red blood cells in their blood. There have been more than 30 different G6PD mutations identified with variable clinical manifestations.

There are many G6PD variants. The normal or wild type is designated as G6PD B. The African variant G6PD A<sup>+</sup> (20% black men) has normal enzyme activity. The G6PD A<sup>-</sup> is an unstable form of A resulting in rapid enzyme degradation and enzyme deficiency as the red blood cells age and is susceptible to hemolysis.

Diagnosis: NADPH production fluorescent screening test, quantitative measurement of NADPH production, and Heinz body test (increased Heinz bodies 3-4 per cells). Bite cells are observed in the peripheral blood, secondary to splenic filtration and elimination of Heinz bodies.

G6PD deficiency is the most common enzymatic deficiency associated with drug-induced hemolysis (see below). G6PD deficiency results in the decreased synthesis of NADPH, which in turn causes a decreased synthesis of reduced glutathione. Glutathione normally protects against oxidant injury by catalyzing the breakdown of oxidant compounds like hydrogen peroxide ( $H_2O_2$ ).

*Drugs and chemicals that may cause hemolytic anemia in G6PD deficiency:*

- Primaquine and chloroquine (antimalarial)
- Sulfonamides (Sulfacetamide, Sulfanilamide, Sulfapyridine)
- Nitrofurantoin
- Acetanilid
- Dimercaptosuccinic acid
- Furazolidone
- Glibenclamide
- Isobutyl nitrite
- Methylene blue
- Toluidine blue
- Nalidixic acid
- Naphthalene
- Niridazole
- Phenazopyridine
- Phenylhydrazine
- Thiazolsulfone
- Urate oxidase.

1. Drugs associated with formation of warm-type autoantibodies: methyl dopa, fludarabine.
2. Drugs absorbed or bound to red blood cell surfaces as haptens: penicillin.
3. Drugs that bind to red blood cell as haptens and form immune complexes: second and third generation cephalosporins (cefotetan is the most common), quinine, quinidine, NSAID.
4. Drugs associated with thrombotic microangiopathies: mitomycin, cyclosporine and tacrolimus.

### *Anemia Associated with Hemoglobinopathies*

Hemoglobinopathies are inherited disorders of hemoglobin synthesis that affect the structure, function, or production of hemoglobin. The laboratory findings and clinical presentations range from asymptomatic laboratory abnormalities, to hemolytic anemia, erythrocytosis, cyanosis and even death. Hemoglobinopathies are the most common inherited red blood cell disorder worldwide. The two most clinically significant hemoglobinopathies are sickle cell disease and thalassemia.

The main function of red blood cells is to carry out the exchange of oxygen and carbon dioxide between the lung and body tissues. Hemoglobin present in the red blood cells is responsible for this gaseous exchange. Erythropoiesis first occurs in yolk sac (first 1-3 months after conception), then in the liver (2-10 months after conception), and then in the spleen (3-8 months after conception). Erythropoiesis in the bone marrow occurs approximately 4 months after conception.

Hemoglobin is composed of four polypeptide chains (Table 2-6). In the embryologic stage, the hemoglobin Gower 1 (two  $\zeta$  and two  $\epsilon$  chains), Portland (two  $\zeta$  and two  $\gamma$  chains), and Gower 2 (two  $\alpha$  and two  $\epsilon$  chains), are formed. In fetus stage, hemoglobin F (two  $\alpha$  and two  $\gamma$  chains) is predominant. In the adult, the hemoglobin molecule HbA is predominant (two  $\alpha$  and two  $\beta$  chains).

**TABLE  
2-6**

**Hemoglobin synthesis and composition at different development stages**

Hemoglobin	Peptide chains	Composition
<b>Embryo</b>		
Gower <sub>1</sub>	(2 $\zeta$ 2 $\epsilon$ )	$\zeta$ equivalent $\alpha$ chain
Portland	(2 $\zeta$ 2 $\gamma$ )	$\epsilon$ equivalent $\beta$ chain
Gower <sub>2</sub>	(2 $\alpha$ 2 $\epsilon$ )	
<b>Newborn</b>		
HbA	(2 $\alpha$ 2 $\beta$ )	25%
HbA <sub>2</sub>	(2 $\alpha$ 2 $\delta$ )	1%
HbF	(2 $\alpha$ 2 $\gamma$ )	75%
<b>Normal adult</b>		
HbA	(2 $\alpha$ 2 $\beta$ )	>95%
HbA <sub>2</sub>	(2 $\alpha$ 2 $\delta$ )	<3.5%
HbF	(2 $\alpha$ 2 $\gamma$ )	1%



## Sickle Cell Disease and other $\beta$ -chain Mutation Hemoglobinopathies (Table 2-7)

Sickle cell anemia is highly prevalent in sub-Saharan and equatorial Africa with a lesser but significant prevalence in the Middle East, India, and the Mediterranean region, closely mirroring the worldwide distribution of falciparum malaria. Sickle cell mutation may relate to resistance against malaria infection.

Sickle cell disease (HbS) is due to a **glutamic acid to valine** substitution at the **6th amino acid** of the  $\beta$ -globin chain of hemoglobin (HbA). This mutation causes the hemoglobin molecule to become insoluble upon deoxygenation. Therefore, red blood cells containing deoxy HbS polymer are rigid which may lead to hemolytic anemia, inflammatory states, and painful vasoocclusive episodes, and multiple organ damage resulting in shortened life expectancy.

There is considerable heterogeneity in the severity of sickle cell disease. For example, elevated levels of hemoglobin F have antisickling effect, and concomitant  $\alpha$ -thalassemia leads to a decrease in hemolysis. Sickle cell disease results from homozygous mutations of both  $\beta$ -globin chains, or from a compound heterozygosity for sickle hemoglobin and  $\beta$ -thalassemia or another  $\beta$ -globin variant such as hemoglobin C, D, E, or O-Arab. Sickle trait is a heterozygous state of sickle hemoglobin (only one  $\beta$ -globin chain contains the mutation), which is clinically asymmetric and benign.

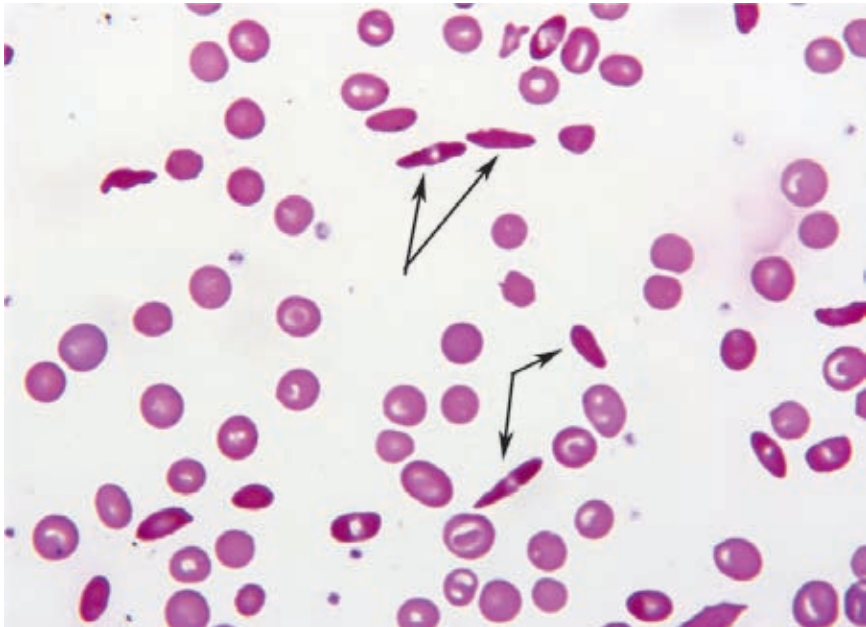
**Sickle cell** patient has an increased risk of **renal medullary carcinoma** (Figs 2-29A and B).

Hemoglobin C (HbC) disease is associated with target cells and spherocytes in the peripheral blood smear, and splenomegaly. HbC disease is due to a **glutamic acid to lysine** substitution at the **6th amino acid** of the  $\beta$ -globin. The peripheral blood smear shows striking red blood cell population of target cells and/or spherocytes, some containing elliptical crystal-like precipitates of hemoglobin C. HbC disease may combine with other hemoglobinopathies such as sickle cell disease or thalassemia (Fig. 2-30).

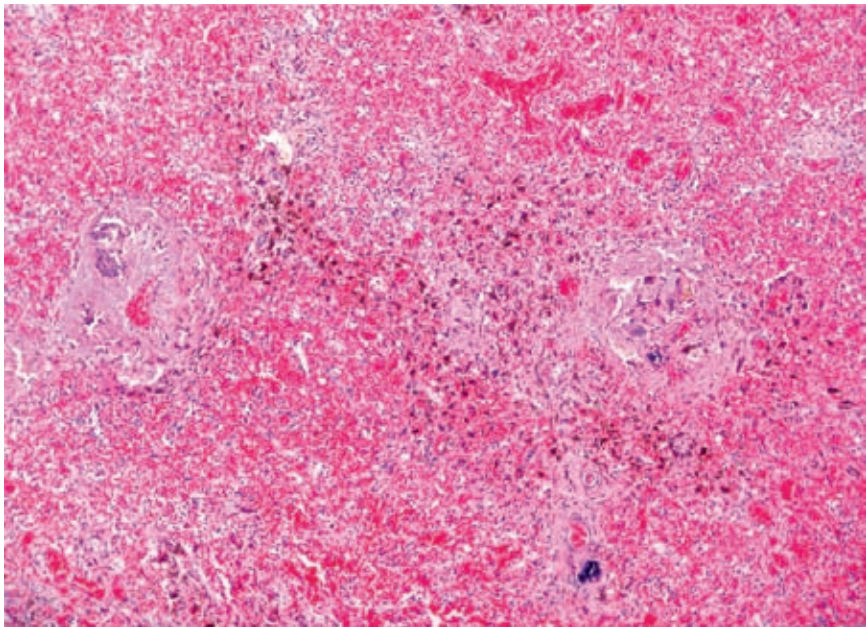
Hemoglobin D (HbD) disease is essentially asymptomatic.

Hemoglobin E (HbE) disease is due to a **glutamic acid to lysine** substitution at the **26th amino acid** of the  $\beta$ -globin. HbE is mildly unstable, but not significantly enough to affect RBC life span. HbE disease is very common in Southeast Asia. The high frequency of the HbE gene may be a result of the thalassemia phenotype associated with its inheritance. Heterozygotes resemble individuals with mild  $\beta$ -thalassemia trait. Homozygotes have somewhat more marked abnormalities but are

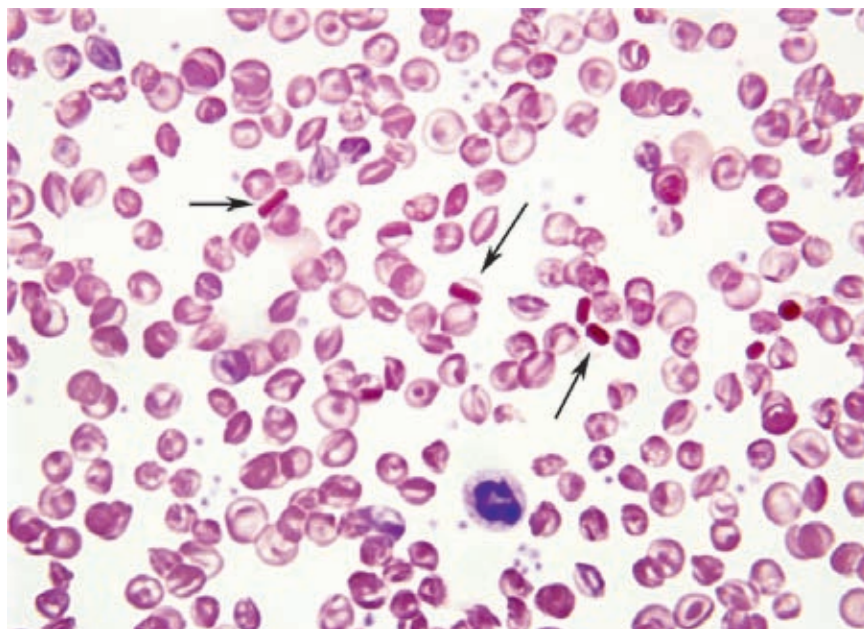




**Fig. 2-29A: Sickle cell disease** showing sickle cells (arrows), hypochromic target cells, and polychromasia (Peripheral blood smear).



**Fig. 2-29B: Sickle cell disease** involving the spleen, showing hemosiderin deposits in macrophages (Gamna-Gandy bodies), sinus congestion and a reduction in both red and white pulp (splenectomy).



**Fig. 2-30: Hemoglobin C disease and thalassemia trait** showing hemoglobin C crystals (arrows) and numerous target cells (Peripheral blood smear).

**TABLE  
2-7**

**$\beta$ -chain mutation and common related hemoglobinopathies**

Disease and mutation	Hemoglobin electrophoresis		
HbS (position 6 Glu $\rightarrow$ Val mutation): Trait (A/S) Disease (S/S)	HbA 60%, No HbA,	HbS 40% HbS>80%,	HbF<20%
HbC (position 6 Glu $\rightarrow$ Lys mutation): Trait (A/C) Disease (C/C)	HbA 60%, No HbA,	HbC 40% HbC>90%,	HbF<10%
HbE (position 26 Glu $\rightarrow$ Lys mutation): Trait (A/E) Disease (E/E)	HbA 60%, No HbA,	HbE 40% HbE>90%,	HbF<10%

asymptomatic. Compound heterozygotes for HbE and a  $\beta$ -thalassemia gene can have  $\beta$ -thalassemia intermedia or  $\beta$ -thalassemia major, depending on the severity of the co-inherited thalassemic gene.

## Thalassemia

The thalassemias are distributed widely in the Mediterranean, the Middle East, India, Pakistan, Southeast Asia, and China.

The clinical presentation of  $\alpha$ - and  $\beta$ -thalassemia varies from normal to early fetal demise. There are two main classes of thalassemia,  $\alpha$  and  $\beta$ , in which the  $\alpha$ - and  $\beta$ -globin genes are involved.

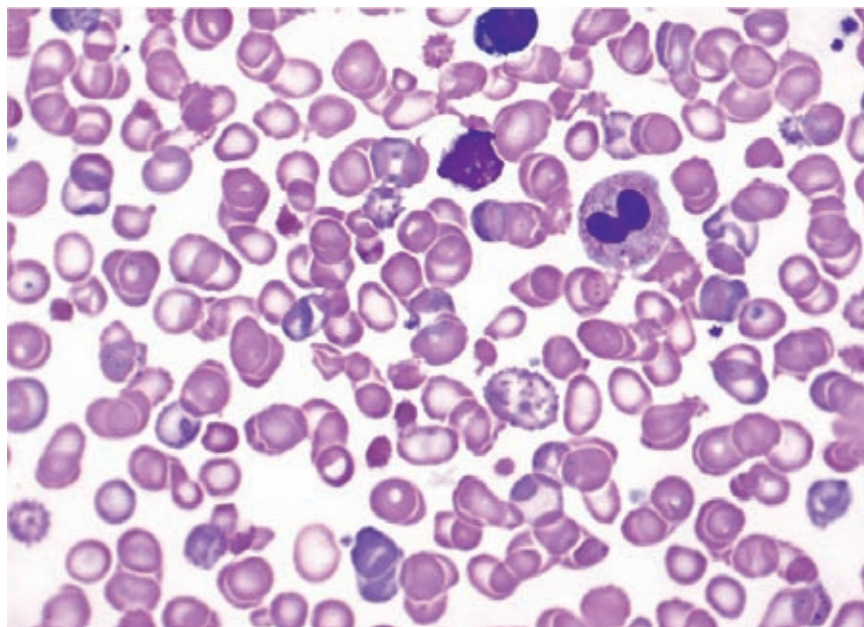
In  $\beta$ -thalassemia, excess  $\alpha$ -chains damage red blood cell precursors and red blood cells resulting in anemia. Anemia, in turn, results in the expansion of the marrow, with severe effects on development, bone formation and growth. The major cause of morbidity and mortality is the result of iron deposition in the endocrine organs, liver, and heart, which is due to increased intestinal absorption of iron and the effects of multiple blood transfusions.

In  $\alpha$ -thalassemias, excess  $\beta$ -chains form hemoglobin H (four  $\beta$ -chains), which is soluble and does not precipitate in the bone marrow. However, hemoglobin H is unstable and precipitates in older circulating red blood cells. Therefore, the anemia of  $\alpha$ -thalassemia is hemolytic rather than dyserythropoietic.

Normal adults have four copies of the  $\alpha$ -globin chain. The  $\alpha$ -thalassemia “silent carrier” patients have a deletion of  $\alpha$ -globin genes, and the patient is clinically and hematologically normal. In the “ $\alpha$ -thalassemia trait”, patients have two  $\alpha$ -globin genes deletions. These patients are clinically normal and have a normal life expectancy and performance status with a mild microcytic anemia. Patients with HbH disease have three  $\alpha$ -globin chain genes deletions (Fig. 2-31) and patients have chronic hemolytic anemia of variable severity with splenomegaly. Laboratory studies show decreased hematocrits (22-32%), and decreased MCV (60–70 fL). The peripheral blood smear shows hypochromia, microcytosis, target cells, poikilocytosis, and an elevated reticulocyte count. Hemoglobin electrophoresis shows the presence of a fast migrating hemoglobin H, which usually comprises 5-30% of the hemoglobin. Supravital dyes on the peripheral blood smear demonstrate the presence of hemoglobin H. Hydrops fetalis (Hb Barts) results from a deletion of all  $\alpha$ -globin chains and is not compatible with life (Table 2-8).

The  $\beta$ -thalassemias are divided into two main categories:  $\beta^0$ -thalassemia with no  $\beta$ -chain production, and  $\beta^+$ -thalassemia with a partial deficiency of  $\beta$ -chain production. The hallmark of  $\beta$ -thalassemia is an elevated level of hemoglobin A<sub>2</sub>.  $\beta$ -thalassemia minor shows microcytic anemia, MCV 60-70 fL, Hb 10-13 g/dL, and a normal or increased RBC count.

$\beta$ -thalassemia major presents in infancy and early childhood with massive hepatosplenomegaly and extreme erythroid hyperplasia in the bone marrow,



**Fig. 2-31: Hemoglobin H disease** showing hypochromasia, microcytosis, target cells, poikilocytosis and polychromasia (Peripheral blood smear).

**TABLE  
2-8**

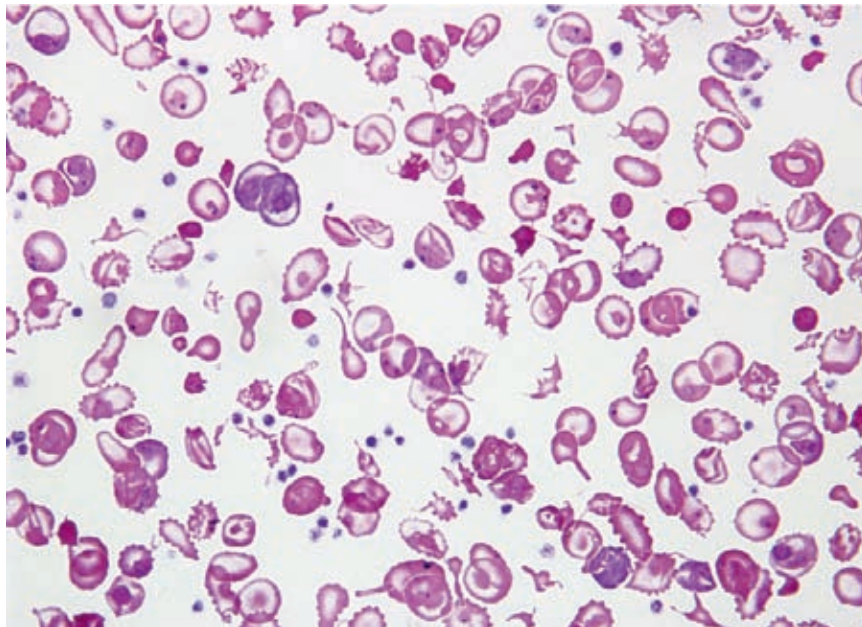
**$\alpha$ -thalassemia**

Phenotype	$\alpha$ chain	Hematologic findings
Normal	$\alpha\alpha/\alpha\alpha$	Normal
Silent carrier	$-\alpha/\alpha\alpha$	Normal
Trait/minor	$-\alpha/-\alpha$ or $-\alpha/\alpha\alpha$	Mild microcytic, hypochromic anemia, normal Hb
HbH disease	$-\alpha/-$	Hemolytic anemia (HbA 70-90%, HbH 5-30%)
Hydrops fetalis (Hb Barts)	$-/-$	Not compatible with life (Hb Barts > 80%, HbH 10-20%, no HbA)

leading to bony deformation, severe hemolytic anemia, and failure to thrive. The peripheral blood smear shows microcytic, hypochromic red blood cells, marked anisochromia, poikilocytosis, anisocytosis, teardrop, oval, elliptical, and fragmented cells (Fig. 2-32).  $\beta$ -thalassemia intermedia is more severe than thalassemia minor but milder than transfusion-dependent thalassemia major (Table 2-9).

The  $\delta$ -thalassemias are characterized by a reduced output of  $\delta$ -chains and hence reduced hemoglobin A<sub>2</sub> (normally two  $\alpha$  chains and two  $\delta$  chains)





**Fig. 2-32:  $\beta$ -thalassemia major** showing hypochromic, microcytic erythrocytes, marked anisopoikilocytosis, anisochromia, and fragmented cells (Peripheral blood smear).

**TABLE  
2-9**

**$\beta$ -thalassemia**

Phenotype	$\beta$ -chain	Hematologic findings		
Minor	$\beta^0/\beta$	HbA 90%,	HbA <sub>2</sub> 3-8%,	+/- HbF
Intermedia	$\beta^+/\beta^+$	HbA 50-70%,	HbA <sub>2</sub> 3-8%,	HbF 20-40%
Major	$\beta^0/\beta^0$	No HbA,	HbA <sub>2</sub> 3-8%,	HbF>90%
$\beta$ = normal gene and normal $\beta$ -globulin chain synthesis $\beta^0$ = total gene deletion, no $\beta$ -globulin chain synthesis $\beta^+$ = partial gene deletion, reduced $\beta$ -globulin chain synthesis				

levels in heterozygotes and an absence of hemoglobin A<sub>2</sub> in homozygotes. When  $\delta$ -thalassemias (decreased A<sub>2</sub> level) are inherited with  $\beta$ -thalassemia minor (increased A<sub>2</sub> level), the level of hemoglobin A<sub>2</sub> may be within the normal range (Table 2-10).

## Hereditary Persistence of Fetal Hemoglobin

**Hereditary persistence of fetal hemoglobin (HPFH)** is a benign condition with an increase of HbF ranging from 15-100%. To distinguish HPFH from

**TABLE  
2-10** **$\delta/\beta$  thalassemia**

Phenotype	$\delta/\beta$ -chain	Hematologic findings		
Minor	$\delta\beta^0/\delta\beta$	HbA 80-90%	HbA <sub>2</sub> 3%	HbF 5-30%
Major	$\delta\beta^0/\delta\beta^0$	No HbA	No HbA <sub>2</sub>	HbF 100%

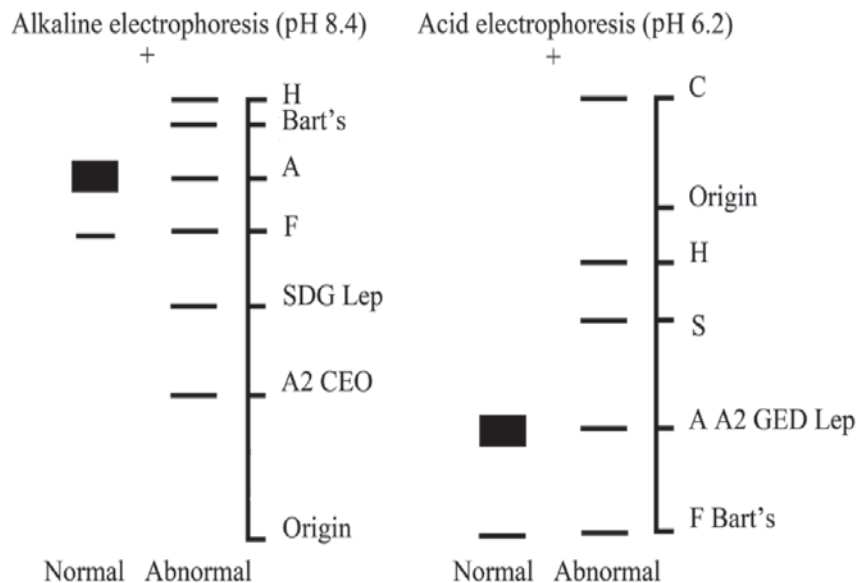
**TABLE  
2-11****Comparison of HbF distribution in  $\beta$ -thalassemia major vs. HPFH**

	Major $\beta$ -thalassemia	HPFH
HbF distribution	Heterogenous	Homogenous

$\beta$ -thalassemia major, compare the HbF distribution in erythrocytes (Table 2-11).

## Methods Used for the Evaluation of Hemoglobinopathies

- 1. Alkaline electrophoresis:** Electrophoresis is carried out at a pH of 8.6, using cellulose acetate or agar as the support medium. Hemoglobin molecules are negatively charged at this pH, so they will move towards the positive terminal (anode) (Fig. 2-33).
- 2. Acid electrophoresis:** Electrophoresis is carried out at a pH of 6.2, using an agar containing agarose as the support medium. Some hemoglobin molecules will form a complex with agarose and will move towards the positive terminal (anode). Those that do not bind agarose will move towards negative terminal (cathode) (Fig. 2-33).
- 3. Isoelectric focusing (IEF)** utilizes small proteins, which are able to carry both current and pH. The proteins will travel to their isoelectric point (net zero charge) and stop at that point.
- 4. High performance liquid chromatography (HPLC)** is used as a rapid screening test for the HbA<sub>2</sub>.
- 5. Globin chain analysis** uses Southern blot, PCR and sequencing to identify globin chain mutations.
- 6. Solubility test for sickling hemoglobins:** Exposure of red blood cell lysate to a reducing agent, if the lysate contains sickling hemoglobins, the solution turbidity will increase. HbS and HbC Harlem are positive, but HbD is negative (they have the same pattern on pH 8.6 electrophoresis).



**Fig. 2-33:** Comparison of normal and abnormal adult hemoglobin alkaline and acid electrophoresis.

7. **Sickle test:** Cells containing HbS may not appear as sickled RBC on a standard blood smear. If the blood is pretreated with sodium metabisulfite before making the smear, it will induce sickling in HbS containing red blood cells.
8. **Kleihauer-Betke stain:** Acid treatment of RBC will elute HbA (ghost cells) and precipitate HbF (dense red blood cells). Flow cytometry can also be used to identify HbF.
9. **Supravital stain:** New methylene blue or brilliant cresyl blue is used to stain HbH and Heinz bodies (unstable hemoglobin precipitate).

## Disorders Related to Heme Synthesis and Iron Overload

### *Porphyrias*

The porphyria is either inherited or acquired disorders. The enzyme activities of the heme biosynthetic pathway are partial or total deficient. Porphyrins and their precursors are toxic. The two main types of inherited defects of porphyrin synthesis associated with light sensitivity and affect the hemopoietic system are congenital erythropoietic porphyria (CEP) and congenital erythropoietic protoporphyria (CEPP).

1. **Congenital erythropoietic porphyria (CEP)** is an inherited autosomal recessive disorder characterized by marked skin photosensitivity and hemolytic anemia. Plasma and erythrocytes contain excessive quantities of uroporphyrin I, coproporphyrin I and protoporphyrin. The deficient enzyme involved in heme synthesis is uroporphyrin III cosynthase. This enzyme defect results in excessive production of uroporphyrinogen I, which form the pigments uroporphyrin I and coproporphyrin I.
2. **Congenital erythropoietic protoporphyria (CEPP)** is an inherited autosomal dominant disorder characterized by mild to moderate photosensitivity without hematologic manifestations. In contrast to CEP, CEPP is relatively common. CEPP results from a deficiency of ferrochelatase activity (less than 50% of normal activity). The deficiency results in an excessive accumulation of protoporphyrin in erythrocytes and massive excretion of protoporphyrin into the stool.

### *Other Anemia-related Syndromes*

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#### **Paroxysmal Nocturnal Hemoglobinuria (PNH)**

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare **acquired stem cell disorder**. PNH is characterized by episodes of hemoglobinuria (brownish urine, particularly upon waking in the morning), thrombocytopenia, leukopenia, and venous thrombosis. The hemoglobinuria is due to complement-mediated hemolysis. This occurs especially at night because the lower oxygen concentration in the blood during sleep increases the susceptibility of red blood cells lysis. Hemolysis occurs because there is a complete or partial **reduce of glycosylphosphatidylinositol (GPI)**, an **anchoring protein** on the erythrocyte membrane surface. These anchoring proteins include complement regulatory proteins **CD55, CD59**, and proteins that are associated with immune function CD58, CD16, and CD14. **Platelet dysfunction** is associated with venous thrombosis, often refractory to thrombolytic therapies and is one of the major causes of death in PNH.

Paroxysmal nocturnal hemoglobinuria is strongly associated with the development of **aplastic anemia** and patients are at an increased risk of developing **acute myeloid leukemia** or myelodysplastic syndrome.

#### **Diagnosis**

1. Urine hemosiderin test: a valuable screening test, since hemosiderinuria is nearly always present in PNH.



2. Sucrose lysis test (Sugar water test): A positive test must be confirmed by acidified serum test or flow cytometry.
3. Acidified serum test (Ham's test) may be used for definitive diagnosis of PNH. The slightly acidic pH promotes complement fixation on the RBCs, RBCs from PNH will be lysed, and the normal RBCs will be resistant to lysis.
4. Flow cytometry detection of CD55 and CD59 on the red blood cells shows a reduced expression in PNH patients.
5. FLAER is another flow-based test that can be used in diagnosis PNH. FLAER is an Alexa488-labeled inactive variant of aerolysin that does not cause lysis of cells. FLAER is more sensitive than CD59 at detecting small abnormal granulocyte populations. However, FLAER cannot be used to assess PNH clones in the erythrocyte lineage, since the latter do not possess surface-bound proteolytic enzymes needed to process the proaerolysin.

### Congenital Dyserythropoietic Anemia (Table 2-12)

The congenital dyserythropoietic anemias are a heterogeneous group of uncommon red blood cell disorders characterized by anemia, multinuclear erythroid precursors in the marrow, ineffective erythropoiesis, and iron overload. Congenital dyserythropoietic anemia is classified as type I, II, and III. Types I and II are inherited as autosomal recessive disorders, and type III disease is inherited as a dominant disorder. Type I disease is caused by mutations of the CDAN1 gene. An abnormal complex carbohydrate is the causative gene in most type II patients. Type III shows a tendency for retinal angioid streaks and is associated with the development of multiple myeloma.

**TABLE  
2-12**

**Comparison of congenital dyserythropoietic anemia type I, II, and III**

	Bone marrow morphology	Acidified serum test	Sugar water test
Type 1	Erythroid megaloblastic changes with internuclear chromatin bridges.	Negative	Negative
Type 2	Binucleated and multinucleated erythroid precursors.	Positive	Negative
Type 3	Predominantly multinucleated erythroid precursors some up to 12 nuclei.	Negative	Negative

## Autoimmune Hemolytic Anemia

Paroxysmal cold hemoglobinuria (PCH) is caused by a biphasic IgG autoantibody called the **Donath-Landsteiner antibody** (a biphasic antibody). The Donath-Landsteiner antibody binds to the P blood group on the RBC surface. On binding, this potent autoantibody fixes complement at low temperatures and activates complement in warm temperature to lyse RBCs, causing clinically significant hemolysis. Symptoms include high fever, chills, headache, abdominal cramps, nausea and vomiting, diarrhea, and leg/back pain that develops with cold exposure (Table 2-13).

**TABLE  
2-13**

**Comparison of warm and cold type autoimmune hemolytic anemia**

	Warm type	Cold type
Immunoglobulin	IgG	IgM
Complete activation	Rare	Yes
PB smear	Spherocytes, Nuc RBC	RBC angulation
Onset	Abrupt	Insidious
Jaundice	Often present	Usually absent
Splenomegaly	Yes	Usually absent
Drug-induced antibody	Common	Rare
Viral or mycoplasma-induced antibody	Rare	Common



CHAPTER

3

# Disorders of Platelets and Coagulation Factors

## Coagulation Factors

### Coagulation Factors Production

All coagulation factors are made by hepatocytes in the liver, except for Factor VIII, which is made by endothelial cells in the liver. The half-lives of these coagulation factors is variable, ranging from 6 hours (Factor VII) to 5 days (Fibrinogen). The synthesis of these coagulation factors depends on the availability of vitamin K.

**TABLE  
3-1**

Site of synthesis and function of coagulation factors

Name	Site of synthesis	Function
Factor I (fibrinogen)	Liver	Converts to fibrin to form clot
Factor II (prothrombin)	Liver	A protease, which activates other factors
Factor V	Liver	Cofactor of X
Factor VII	Liver	Protease, activates IX, X
Factor VIII	Liver (endothelial cells)	Cofactor of IX
Factor IX	Liver	Protease, activates X
Factor X	Liver	Protease, activates II, form complex with V
Factor XI	Liver	Protease, activates IX
Factor XII	Liver	Protease, activates XI, VII and prekallikrein
Factor XIII	Liver	Transamination, cross-link fibrin
Von Willebrand factor	Endothelial/Megakaryocytes	Cofactor, binds to VIII
Tissue factor	Many cells	Cofactor of VIIa

A deficiency of a coagulation factor may cause bleeding problems **except for XII (Hageman factor), prekallikrein, and high molecular weight kininogen**. Some of the coagulation factors are also **acute phase reactants** including VIII, VWF, and fibrinogen.

### Vitamin K-dependent Coagulation Factors

The vitamin K-dependent coagulation zymogens are precursors of serine proteases that must be proteolytically activated to express their enzymatic activity. They all share a similar protein domain structure.

Both protein C and protein S are glycoprotein that undergoes vitamin K-dependent posttranslational modification.

Vitamin K-dependent coagulation factors including:

1. Prothrombin (factor II)
2. Factor VII
3. Factor IX (deficiency in hemophil B)
4. Factor X
5. Protein C
6. Protein S

Except **Protein S**, all the above are **proteinase** zymogens.

### Coagulation Tests

1. **PT (Prothrombin time assay):** Screens for abnormalities in both the extrinsic pathway (Factor VII) and in the common pathway (Factors X, V, II, I). It does not assess the intrinsic pathway (Factors XII, XI, IX, VIII). Of note, Factor XIII is not measured by either of the coagulation assays, PT or aPTT. Increased PT indicates decreased synthesis of the vitamin K-dependent clotting factors (Factors II, VII, IX, X), or decreased levels of Factor V or fibrinogen. The PT is also used to monitor Warfarin (Coumadin) therapy, and vitamin K deficiency (liver disease). The PT is only prolonged when a factor level is decreased by 30-40% of normal. The normal reference range is 12-14 seconds.
2. **aPTT (active partial thromboplastin time):** Screens for abnormalities in both the intrinsic pathway (Factors XII, XI, IX, VIII), and the common pathway (Factors X, V, II, I). To summarize, the aPTT measures all coagulation factors, except Factors VII and XIII (which are not measured by either PT or aPTT). The aPTT is also used to monitor heparin therapy, and as screen for lupus anticoagulant. The aPTT is only prolonged when a factor level is decreased by 30-40% of normal. The normal reference range is 25-40 seconds.

**TABLE  
3-2**

aPTT and PT used to screen for inherited bleeding disorders

aPTT	PT	Possible disorders
Normal	Normal	XIII, platelet, VWF, $\alpha_2$ -antiplasmin
Increased	Normal	VIII, IX, XI
Increased	Increased	V, X, II (prothrombin), $\alpha_2$ -antiplasmin
Normal	Increased	VII

**International normalized ratio (INR)** used to adjust for individual laboratory variation in the PT, and calculated as following:

$$INS = \left( \frac{PT_{pt}}{PT_{ref}} \right)^{ISI}$$

PT<sub>pt</sub>: patient PT

PT<sub>ref</sub>: normal mean PT

ISI: International sensitivity index is usually supplied by manufacture.

3. **TT (thrombin time):** Screens for abnormalities in the conversion of fibrinogen to fibrin.
4. **Reptilase time:** Reptilase time measures the conversion of fibrinogen to fibrin but is insensitive to heparin. The finding of prolonged thrombin time values and a normal reptilase time suggests contamination of the sample by heparin.
5. **Bleeding time:** Previously used to screen for inherited platelet dysfunction, but now has limited use.
6. **Mixing study**  
Mixing study detects the presence of serum antibodies that are neutralizing the activity of coagulation factors. The patient's plasma is mixed in equal volume with a normal plasma specimen and aPTT assay is performed immediately (zero time), and 1-2 hours later at 37° C (98.6° F). The presence of heparin in the sample can be verified by the finding of a prolonged thrombin time. Toluidine blue or other agents may be used to neutralize heparin. In lupus anticoagulant, the PT usually is less prolonged than aPTT (Table 3-3).
7. **Dilute Russell's viper venom time (DRVVT):** Identifies the presence of lupus anticoagulant. DRVVT is a modified PT assay; the clotting is initiated by venom and phospholipid. The venom bypasses factor VII, and directly converts Factor X to Xa. This test can also be used to detect a Factor VII deficiency.

**TABLE  
3-3**

**Interpretation of mixing study**

Prolonged aPTT at	Time 0	Time 1-2 hours	Interpretation
	Corrected	Corrected	Suggests factor deficiency
	Not corrected	Not corrected	Suggests lupus anticoagulant or heparin present
	Corrected	Not corrected	Suggests antibody inhibitor (such as anti-VIII antibodies)



### *The Natural Inhibitors of Coagulation*

Several physiologic antithrombotic factors exist under normal circumstances to prevent clotting (Table 3-4).

**TABLE  
3-4**

**The natural inhibitors of coagulation**

Name	Site of synthesis	Function
Protein C	Liver	Protease that inactivates factor Va, VIIIa
Protein S	Liver	Cofactor for activated protein C
Antithrombin III	Liver	Inhibits factor IIa (thrombin), Xa, and others
Tissue factor pathway inhibitor (TFPI)	Many cells	Down-regulates VII-tissue factor pathway
Heparin cofactor II	Liver	Inhibitor of thrombin

- 1. Antithrombin (or antithrombin III, AT)** is a serine protease inhibitor, which neutralizes numerous coagulation proteases. AT is the major inhibitor of thrombin (Factor IIa). AT also inhibits Factor Xa (common pathway), and Factors IXa, XIa, XIIa (intrinsic pathway). Inherited deficiencies of AT results in a life-long predisposition to venous thromboembolism. AT, which is normally activated by heparin-like molecules, is the primary target for heparin-based anticoagulant therapy. AT activity, and thus heparin-based therapy, is monitored by the aPTT.

Family of serine protease inhibitors:

1. Antithrombin III (greatly enhanced function in the presence of heparin)
  2.  $\alpha_1$ -antitrypsin
  3.  $\alpha_2$ -antiplasmin
  4. Heparin cofactor II
  5. Plasminogen activator inhibitor
- 2. Protein C** is activated by thrombin and the cofactor thrombomodulin to become activated protein C (APC). APC acts as an anticoagulant by inactivating factors Va and VIIIa. Quantitative deficiencies or qualitative deficiencies, or resistance to the action of activated protein C, Factor V Leiden mutation lead to hypercoagulable states.
  - 3. Protein S**, a single-chain plasma glycoprotein, is a cofactor for activated protein C. Protein S depends on vitamin K for its synthesis. Unlike the other vitamin K-dependent factors, it does not contain a serine protease

domain and so does not have the potential to catalyze reactions. Quantitative deficiencies or qualitative deficiencies of protein C lead to hypercoagulable states.

4. **Tissue factor pathway inhibitor (TFPI)** is a plasma protease inhibitor that regulates the TF-induced extrinsic pathway of coagulation. TFPI inhibits the TF/FVIIa/FXa complex, and inactivates the TF/factor VIIa initiation of coagulation. Heparin promotes the release of TFPI from endothelial cells.

### *Fibrinolytic System*

During the normal coagulation cascade, fibrinogen (Factor I) is converted to Fibrin (Factor Ia) which is then cross-linked and becomes part of a stable platelet-fibrin thrombus. The fibrinolytic system prevents the overproduction of intravascular thrombi. The key members of the fibrinolytic system are tissue plasminogen activator (tPA), plasminogen, plasmin, and  $\alpha_2$ -antiplasmin,.

Tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) convert plasminogen into its active form, plasmin. Plasmin cleaved fibrin into fragments and releases fibrin split products (FSP) and D-dimers. D-dimers are a sensitive serum marker of clot formation, thus providing evidence of an activated coagulation cascade. The D-dimer assay is used for the evaluation of deep venous thrombosis (DVT) and pulmonary embolism (PE).

The fibrinolytic activity is regulated by plasminogen activator inhibitors (PAI-1 and PAI-2), which inhibits tPA and uPA, and by  $\alpha_2$ -antiplasmin, which degrades plasmin (Table 3-5).

**TABLE  
3-5**

**Fibrinolytic system**

	Site of synthesis	Function
Plasminogen	Liver	Precursor of plasmin
Plasmin	Liver	Serine protease
Tissue plasminogen activator (t-PA)	Endothelial	Activates plasminogen
Streptokinase (SK)	Recombinant	Activates plasminogen
Plasminogen activator inhibitor (PAI-1)	Endothelial, platelet	Inhibitor
$\alpha_2$ -antiplasmin	Liver	Inactivates plasmin

### *Inherited Coagulation Disorders*

All of the factor deficiencies, listed below, with the exception of Hemophilia A and B, are autosomal recessive (AR) inherited disorders. Hemophilia A and B are both X-linked recessive inherited disorders. These factor deficiencies will present clinically with a prolonged aPTT, with the exception of Factor VII and Factor XIII deficiencies. Factor VII deficiency will present with a prolonged PT, and Factor XIII deficiency will not be detected by routine laboratory screening tests.

- Factor II (prothrombin) deficiency:** A rare autosomal recessive disorder and is characterized by a mild to moderate risk of bleeding. Treatment is prothrombin complex concentrates (preferred) or fresh frozen plasma (FFP).
- Factor V deficiency:** An autosomal recessive disorder, which has a moderate risk for bleeding in homozygote and compound heterozygote inherited states. Inherited Factor V deficiency must be distinguished from both an inherited combined deficiency of Factors V and VIII, and an acquired deficiency of Factor V (Table 3-6). Treatment is fresh frozen plasma (FFP).

**TABLE  
3-6**

**Comparison of inherited factor V deficiency, combined deficiency of factor V, VIII, and acquired factor V deficiency**

	PT	aPPT	Factor VIII level	Liver disease or DIC
Inherited V deficiency	Prolonged	Prolonged	Normal	Absent
Inherited combined V and VIII deficiency	Prolonged	Prolonged	Low	Absent
Acquired V deficiency	Prolonged	Prolonged	Normal or low	Present

- Factor VII deficiency:** A rare autosomal recessive disorder, which is symptomatic mainly in cases of homozygotes and compound heterozygotes inherited states. The symptoms vary from mild to severe bleeding. Factor VII deficiency is the only coagulation disorder that produces a prolonged PT with normal aPT.
- Factor VIII deficiency (hemophilia A) and IX deficiency (hemophilia B or Christmas disease)** are two of the most clinically important factor deficiencies. They both result from an X-linked recessive pattern of inheritance. The severity of disease depends on the levels of

circulating Factor VIII:C and Factor IX, respectively. The normal adult reference ranges for both Factors VIII:C and IX activity level is 50-150% (50-150 IU/dL). Factor levels 5-30% are classified as mild hemophilia, factor levels of 1-5% are classified as moderate hemophilia, and factor levels less than 1% are classified as severe hemophilia. Of note, patients with nephrotic syndrome may develop an acquired form of Factor IX deficiency. Treatment of Factor VIII and Factor IX deficiencies is through administration of recombinant factor concentrates.

5. **Factor X deficiency:** An autosomal recessive disorder, which has a moderate to severe risk of bleeding. Factor X deficiency usually presents with a prolonged aPTT and PT, and a prolonged Russell viper venom time. Of note, patients with systemic amyloidosis may develop an acquired form of Factor X deficiency. Treatment of Factor X deficiency is fresh frozen plasma (FFP).
6. **Factor XI deficiency:** An autosomal recessive disorder, which has a mild to moderate risk of bleeding, usually presents after an injury. Treatment is fresh frozen plasma (FFP).
7. **Deficiencies of factor XII, prekallikrein, and high molecular-weight kininogen:** Factor XII (Hageman Factor) deficiency is a rare autosomal recessive disorder, which results in a prolonged aPTT, however, it is not associated with any clinical bleeding or surgical hemorrhagic risk. Prekallikrein and high-molecular-weight kininogen (HMWK) deficiencies share similar clinical features with Factor XII deficiency. Prekallikrein and HMWK deficiencies result in a prolonged aPTT, but are not associated with any risk of clinical bleeding.
8. **Factor XIII (fibrin-stabilizing factor) deficiency:** An autosomal recessive inherited disorder, which results in a mild risk of bleeding. Diagnosis of a Factor XIII deficiency is often made at birth, by the observation of bleeding from the umbilical cord stump. The normal function of Factor XIII is to crosslink strands of fibrin and stabilize the fibrin clot. Deficiency of this factor results in unstable blood clots that are susceptible to fibrinolytic degradation by plasmin. All routine laboratory screening tests are normal. Diagnosis of Factor XIII deficiency relies on the 5 mol/L urea lysis test, in which a positive test shows a blood clot dissolving within 24 hours. Treatment is fresh frozen plasma (FFP).
9.  **$\alpha_2$ -Antiplasmin deficiency:** A rare disorder, which results in inadequate inhibition of plasmin, and in turn, resulting in increased fibrinolysis. Heterozygote inheritance usually results in a mild bleeding disorder, but homozygote inheritance may result in a severe bleeding disorder.

All of the routine laboratory screening tests will be normal, but the antiplasmin level will be decreased. Treatment is fresh frozen plasma (FFP) and anti-fibrinolytic drugs.

- 10. Afibrinogenemia:** A rare autosomal recessive inherited disorder, which may result in severe bleeding. Diagnosis is often at birth, observed by bleeding from the umbilical cord stump. Laboratory screening tests will show prolonged aPTT and PT, prolonged thrombin time, and a marked decrease in fibrinogen. Treatment is cryoprecipitate (preferred) or FFP.

### *Acquired Coagulation Disorders*

Acquired coagulation disorders may be due to decreased production (liver disease), increased consumption (DIC), or immune-mediated, related to the presence of an autoantibody against a specific clotting factor.

#### **Liver Disease and Vitamin K Deficiency**

The evaluation of liver disease is an important part of the clinical workup for a patient with an acquired coagulation disorder. Impaired hepatic function (hepatitis, cirrhosis) leads to decreased production of several coagulation factors, including Factors II, V, VII, IX, fibrinogen, protein C and S. In the clinical scenario of hepatitis, Factor VIII levels may be elevated due to the fact that Factor VIII is an acute-phase reactant.

Vitamin K participates in posttranslational gamma-carboxylation of the factors II, VII, IX, and X which is necessary for their activity.

#### **Disseminated Intravascular Coagulopathy (DIC)**

DIC results from uncontrolled local or systemic activation of coagulation, leading to the activation and consumption of platelets, coagulation factors, and fibrinogen.

Disease that is commonly associated with DIC:

1. Metastatic carcinoma (adenocarcinoma)
2. Tissue injury
3. Acute promyelocytic leukemia (APL)
4. Infections (Gram-negative endotoxemia, Gram-positive septicemia, Rocky mountain spotted fever, viral)
5. Obstetric disorders (amniotic fluid embolism, retained fetus, placenta abruption).

## **Antibodies to Coagulation Factors (Inhibitors to Coagulation Factors)**

Antibodies to specific coagulation factors may develop after a patient receives factor replacement therapy, or they may occur spontaneously. Factor VIII is the most common immune-mediated Factor deficiency. Acquired immune-mediated deficiencies of Factor II, V, IX, and XI are also reported.

### ***Lupus Anticoagulant Antibody***

Lupus anticoagulant antibody is the most common antibody against factor VIII and V. The presence of lupus anticoagulant may be associated with venous or arterial thrombotic disease or bleeding due to the presence of antibodies to prothrombin.

Several coagulation tests are available for detecting lupus anticoagulant antibody.

1. **Dilute Russell viper venom time (DRVVT)** is considered one of the most sensitive tests. The assay uses Russell viper venom (RVV) in a system containing limiting quantities of diluted rabbit brain phospholipid. RVV directly activates coagulation factor X, leading to formation of fibrin clot. Lupus anticoagulant antibody prolongs DRVVT by interfering with assembly of the prothrombinase complex; however, the prolongation is reversed by adding excess phospholipid to the reaction. To ensure that prolongation of the clotting time is not a result of a factor deficiency, the procedure includes mixture of patient and control plasmas. Anticoagulant therapy with heparin, warfarin, or direct thrombin inhibitors may yield falsely abnormal test results.
2. **Activated partial thromboplastin time test (aPTT):** Lupus anticoagulant antibody frequently causes prolonged aPTT in otherwise healthy individuals. The lupus anticoagulant antibody should be suspected if a prolonged aPTT is not corrected by a mixing study.
3. **The Kaolin clotting time (KCT)** is essentially an activated partial thromboplastin time test (aPTT), without added phospholipids. The test relies on residual cell membrane fragments and plasma lipids to provide a phospholipid surface for coagulation reactions. Phospholipid is believed to be the main target of antiphospholipid antibodies, which are often associated with lupus anticoagulants. If lupus anticoagulants are present, KCT will be prolonged.
4. **Hexagonal phase phosphatidylethanolamine (HPE) test:** HPE neutralizes lupus coagulant present in the patient plasma, resulting in a



shortened clotting time versus the same patient plasma without added HPE. A positive result also confirms the phospholipid-dependent nature of the detected antibody.

To distinguish acquired inhibitors from lupus anticoagulants, the dilute Russell's viper venom test (DRVVT) and the hexagonal-phase phospholipids test will be negative in patients with an acquired inhibitor and positive in patients with lupus anticoagulants. Lupus anticoagulant usually interferes with many coagulation factors (VIII, IX, XII, and XI); whereas acquired inhibitors are usually affect a single factor (Table 3-7).

**TABLE  
3-7****Lupus anticoagulant vs VII factor deficiency**

	<b>PT</b>	<b>DRVVT</b>
Lupus anticoagulant	Normal	Prolonged
VII deficiency	Prolonged	Normal

### ***Criteria for the Laboratory Diagnosis of the Lupus Anticoagulant***

1. Prolongation of at least 1 phospholipid-dependent coagulation test with the use of platelet poor plasma (aPTT, DRVVT, KCT).
2. Failure to correct the prolonged coagulation time by mixing patient and normal plasma.
3. Confirmation of the lupus anticoagulant by demonstrating correction of the prolonged coagulation time by addition of excess phospholipid or freeze-thawed platelets.
4. Exclusion of alternative coagulopathies (Factor VII, Factor V antibodies).

### **Acquired Hemophilia A**

Acquired hemophilia A is associated with acquired (spontaneous) factor VIII antibodies. Affected patients usually present with spontaneous bleeding and more likely to have a more severe risk of bleeding than patients with inherited hemophilia A. Approximately half of these patients have an underlying condition (autoimmune disorder, malignancy, pregnancy, etc.) and the rest of the cases are idiopathic. Acquired factor VIII inhibitors sometimes resolve spontaneously.

### Primary Amyloidosis

Systemic amyloidosis may lead to an acquired Factor X deficiency, which results in severe bleeding. In this disorder, Factor X binds to circulating amyloid fibrils, leading to inactivation and rapid clearance of Factor X from the blood.

### Nephrotic Syndrome

Thromboembolic complications are a major concern for patients with the nephrotic syndrome. Renal vein thrombosis, pulmonary embolism and deep vein thrombosis are frequently seen in patients with membranous glomerulonephritis. Arterial thrombosis is less common, but is a serious complication causing morbidity.

Patients with nephrotic syndrome have increased levels of coagulation factors V, VII, VIII, VWF, and fibrinogen, in addition to decreased levels of protein S, plasminogen, Factors IX, XII, and antithrombin III (ATIII).

### Hodgkin Lymphoma

Patients with Hodgkin lymphoma, may also develop an acquired factor deficiency, usually either Factor VII and/or Factor XII.

### *Platelets and von Willebrand Factor*

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Platelets are important mediators of hemostasis. Their membranes contain receptors for collagen, ADP, von Willebrand Factor, and fibrinogen.

Platelets contain **three types of granules**:

1. **Lysosomes**: acid hydrolase
2.  **$\alpha$ -granule**: Platelet factor 4,  $\beta$ -thromboglobulin, PDGF, fibrinogen, VWF, and Factor V.
3. **Dense body ( $\delta$ -granule)**: ATP, ADP, calcium, and serotonin.

Endothelial cells and megakaryocytes synthesize von Willebrand Factor (VWF), a high molecular weight glycoprotein. VWF serves as a carrier for Factor VIII (**VWF:C**) and as an adhesive link between platelets and the injured blood vessel wall. VIII:Ag is the quantity of VIII: C. VWF is stored in  $\alpha$ -granules of the platelet.

When a blood vessel wall is injured, platelets adhere to the exposed collagen and von Willebrand Factor. Bindings results in platelet activation, which releases the contents of their granules. The released ADP acts on the

ADP receptors in the platelet membranes to produce further accumulation of more platelets (platelet aggregation).

## Thrombocytopenia

Clinically significant thrombocytopenia is usually less than  $50 \times 10^3/\mu\text{l}$ , serious hemorrhage should not occur unless the platelet count is less than  $20 \times 10^3/\mu\text{l}$ .

### Causes:

#### 1. Acquired Thrombocytopenia

- Failure of marrow production (i.e. tumor, fibrosis, MDS).
- Increase platelet destruction (i.e. ITP, TTP, DIC, post transfusion, SLE).
- Splenic sequestration.
- Heparin-induced thrombocytopenia (binds platelet factor 4 to form immunocomplex).
- Others: hemodilution, EDTA-pseudothrombocytopenia.

#### 2. Inherited Thrombocytopenia (Table 3-8)

**TABLE  
3-8**

**Inherited thrombocytopenias**

Disease	Inheritance	Morphology	Abnormality
Bernard-Soulier syndrome	Autosomal recessive	Giant platelet	Absent GPIb/V/IX
Gray platelet syndrome	Autosomal recessive	Large platelet	Absent $\alpha$ granule
May-Hegglin anomaly	Autosomal dominant	Large platelet	Inclusion (Döhle body)
Wiskott-Aldrich syndrome	X-linked	Small platelet	Immunodeficiency and deficient of platelet calpain

#### 3. Other syndromes/disorders associated with **large platelets**:

- Epstein syndrome (autosomal dominant)
- Fechtner syndrome (autosomal dominant)
- Sebastian syndrome (autosomal dominant)
- Montreal platelet syndrome (autosomal dominant)
- Hereditary macrothrombocytopenia (autosomal dominant).

## Von Willebrand Disease

Von Willebrand disease (VWD) is the most common inherited bleeding disorder. VWD is subdivided into three general categories (Types 1, 2 and

**TABLE  
3-9****Von Willebrand disease (VWD)**

Type	Comments
Type 1	Quantitative deficiency reduced production of VWF. VWF is normal in structure and function, normal multimer pattern, but decreased in quantity. Responds to DDAVP therapy
Type 2A	Qualitative defect, loss of functional VWF multimers (intermediate and high molecular weight). Responds to DDAVP therapy
Type 2B	Qualitative defect, loss of high molecular weight multimers. Due to mutations in a segment of VWF that is critical for binding to the platelet glycoprotein Ib (GPIb) receptor, these mutations result in spontaneous binding of VWF to platelets, and subsequent clearance of this complex. <b>DOES NOT respond to DDAVP therapy</b>
Type 2M	Mutations in VWF, decreased interaction of VWF and platelet. Multimer analysis is normal
Type 2N	Mutations within the factor VIII binding domain of VWF, which decreases binding of factor VIII to VWF. Multimer analysis is normal
Type 3	Quantitative defect, multimer is totally absent
Platelet type (pseudo-VWD)	Platelet defect that phenotypically mimics VWD; mutations within the GPIb receptor results in decreased platelet–VWF interactions

3), with Type 2 having additional sub-types. All types of VWD are due to autosomal dominant mutations, except for Type 2N and Type 3, which are due to autosomal recessive mutations (Table 3-9).

TTP is a disorder associated with deficiency or autoantibody to **metalloproteinase (ADAMTS-13)** which cleave unusually large VWF multimer and result in increased numbers of circulating large VWF multimers.

Factor VIII and VWF are acute phase reactants; therefore, a transient elevation of Factor VIII is usually accompanied by elevated VWF level. VIII:Ag is usually decreased in VWD and the ratio of VIII/VIII:Ag is usually decreased in hemophilia A.

## Additional Platelet Disorders

### *Abnormalities of Platelet Glycoprotein Adhesion Receptors*

#### 1. Glanzmann Thrombasthenia

Glanzmann thrombasthenia is a rare autosomal recessive hemorrhagic disorder, due to a defect of platelet glycoprotein IIb (CD41) and/or IIIa (CD61). These

patients present with a low-platelet-type of clinical bleeding, despite a normal platelet count.

The clinical presentation includes menorrhagia, purpura, petechiae of the face and subconjunctival hemorrhage, epistaxis, gingival and gastrointestinal bleeding. Hemarthroses are very rare, which is a distinguishing feature of Glanzmann thrombasthenia from the hemophilias.

Laboratory findings include normal platelet counts, normal platelet morphology, prolonged bleeding times, decreased or absent clot retraction, and abnormal ADP, epinephrine, and collagen platelet aggregation (Table 3-10)

## 2. Bernard-Soulier Syndrome

Bernard-Soulier syndrome is a rare autosomal recessive disorder involving the platelet GPIb/IX/V complex characterized by thrombocytopenia, giant platelets, and a failure of the platelets to bind GPIb ligands of von Willebrand factor and thrombin.

**TABLE 3-10** Platelet aggregation study

Disorder	ADP	Epinephrine	Collagen	Ristocetin
VWD	Normal	Normal	Normal	Absent
VWD 2B	Normal	Normal	Normal	Hyper-responsiveness to low concentration
Bernard-Soulier syndrome	Normal	Normal	Normal	Absent
Glanzmann's thrombasthenia	Absent	Absent	Absent	Normal
Storage pool defect	Absent 2nd	Absent 2nd	Normal or absent 2nd	Normal
Release defect (aspirin or aspirin like)	Absent 2nd	Absent 2nd	Normal or absent 2nd	Normal
Afibrinogenemia (AR)	Normal	Normal	Absent	Normal, absent in low concentration

Absent 2nd = Absent second wave

Epistaxis is the most common clinical presentation of Bernard-Soulier syndrome, followed by ecchymoses, menometrorrhagia, gingival hemorrhage, and gastrointestinal bleeding.

Laboratory findings include thrombocytopenia, large platelets (on peripheral blood smear), prolonged bleeding time, and abnormal platelet aggregation responses.

The hallmark finding of Bernard-Soulier syndrome is the failure of platelets to aggregate in response to ristocetin (Table 3-10).

### **3. Platelet-Type (Pseudo-) von Willebrand Disease**

Platelet-type von Willebrand disease or pseudo-von Willebrand disease is characterized by variable thrombocytopenia, mild to moderate mucocutaneous hemorrhage, prolonged bleeding time, large platelets (on peripheral blood smear), and diminished plasma high-molecular-weight von Willebrand factor multimers (same as type 2B von Willebrand disease). It is due to a defect of platelet GPIb/IX, not von Willebrand factor.

The most characteristic laboratory finding is enhanced platelet aggregation in response to low concentrations of ristocetin (0.5 mg/ml or less).

### ***Platelet Storage Pool Disorder***

Platelet storage pool disorders are a group of inherited platelet disorders, characterized by defects in the release of alpha and/or delta platelet granules. Evaluation, by electron microscopy, demonstrates decreased or absent platelet granules. Bleeding symptoms are variable, but usually mild.

Inherited platelet storage pool disorders include:

1. Chédiak-Higashi syndrome
2. Thrombocytopenia with absent radii
3. Wiskott-Aldrich syndrome
4. Hermansky-Pudlak syndrome.

### ***Hereditary Thrombotic Disorders and Anticoagulant Therapy Monitoring (Table 3-11)***

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The two most common hereditary thrombotic disorders in Caucasians are activated protein C resistance (APC resistance, a Arg506 to Gln mutation in the Factor V gene, also called **Factor V Leiden**) and prothrombin point mutation (single-nucleotide mutation, G20210A, resulting in elevated plasma prothrombin levels). Other common hereditary thrombotic disorders are



**TABLE  
3-11****Anticoagulant therapy monitoring**

Anticoagulant	Test
Unfractionated heparin therapy	aPTT (use Anti-Xa assay if lupus anticoagulant is present)
Low-molecular-weight heparin	Anti-factor Xa assay
Warfarin	PT
Fibrinolytic (Streptokinase, Urokinase t-PA)	Thrombin time
Aspirin	Platelet aggregation test

hyperhomocysteinemia and increased plasma levels of Factor VIII. Hereditary thrombotic disorders due to genetic defects of the anticoagulant proteins, protein C, protein S, and antithrombin are less common. These genetic defects either enhance procoagulant reactions or weaken anticoagulant mechanisms. Venous thrombosis is the most common, but arterial thrombosis can occur (Table 3-12).

**TABLE  
3-12****Hereditary thrombotic disorders**

Disorder	Inheritance	Site of Thromboembolism
Antithrombin deficiency	Autosomal dominant	Venous, heparin-resistant
Protein C deficiency	Autosomal dominant	Venous
Protein S deficiency	Autosomal dominant	Venous
Factor V mutation	Autosomal dominant	Venous and arterial
Prothrombin mutation	Autosomal dominant	Venous
Dysfibrinogenemia	Autosomal dominant	Venous and arterial
Homocystinemia	Autosomal recessive	Arterial and venous

Laboratory assays are widely available to identify most hereditary thrombotic disorders. It is important to identify these disorders, as these disorders will affect patient management during pregnancy, anticoagulant therapy and using of hormones.

### Unfractionated Heparin and Low-Molecular-Weight Heparin (LMWH)

Activated partial thromboplastin time (aPTT) is commonly used for monitoring the anticoagulant effect of heparin. Alternatively, anti-Xa levels may be used for monitoring patients who have prolongation of the aPTT due to lupus anticoagulant.

**Protamine sulfate** can be used to neutralize heparin; protamine is routinely used to reverse the effects of heparin following cardiopulmonary bypass surgery.

LMWH is produced by decreasing the size of the polysaccharide chains. The antithrombotic effect of LMWH is through interactions with antithrombin. Antithrombin inactivates Factor Xa in the same way as unfractionated heparin, but has less effect on thrombin because the shorter polysaccharide length.

### Warfarin

Warfarin is an antagonist of vitamin K. Coagulation Factors II, VII, IX, X, and anticoagulant proteins C and S are synthesized mainly in the liver and are biologically inactive. These coagulation factors are activated by carboxylation that requires the presence of vitamin K.

The INR calculated from the patient's PT is used to monitor efficacy and compliance of warfarin. To monitor warfarin therapy, the patient's PT is determined along with that of a sample of normal pooled plasma. PT measurements are converted to INR measurements. For most indications, the target INR is 2 to 3.

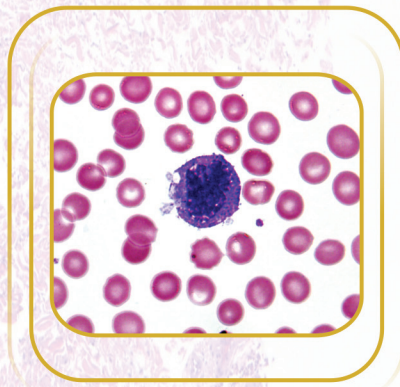
Bleeding is the major toxicity of warfarin, birth defects and skin necrosis are rare complication of using warfarin.

Concurrent administration of warfarin with agents such as alcohol, macrolides, sulfonamides, azole antifungals, and amiodarone (Cordarone) may significantly increase the anticoagulation activity by displacing warfarin from plasma proteins. Chronic alcohol ingestion, nafcillin, barbiturates, and rifampin may significantly decrease the anticoagulation activity.

CHAPTER

4

# Disorders of Nonneoplastic White Blood Cells



## Neutrophil Disorders

### Neutrophilia and Neutropenia

The normal absolute neutrophils count is 1500-7500/ $\mu$ l ( $1.5-7.5 \times 10^9$ /L).

#### Neutrophilia

**Neutrophilia** refers to an absolute neutrophil count (total leukocyte count per microliter  $\times$  percent of neutrophils) that is greater than two standard deviations above the mean of normal individuals (Table 4-1).

<b>TABLE 4-1</b> <b>Diagnostic criteria of neutrophilia</b>	
Age group	absolute neutrophil count
Birth	>28,000/ $\mu$ l ( $28 \times 10^9$ /L)
Infant	>10,000/ $\mu$ l ( $10 \times 10^9$ /L)
Children	>8000/ $\mu$ l ( $8 \times 10^9$ /L)
Adult	>7000/ $\mu$ l ( $0.7 \times 10^9$ /L)

Under normal circumstances, neutrophils follow an orderly progression from the marrow pool (mitosis, maturation and storage) through the blood pool (circulating and marginated pool), then to tissue sites of utilization. **Demargination** refers to a shift of neutrophils from the marginated pool (in the pulmonary vasculature) to the circulating pool. Demargination, noted by the subsequent elevation of the WBC count, may be a feature of steroid therapy, as cortisol disrupts the leukocyte adhesion of the neutrophils to the endothelium of the pulmonary vasculature.

Neutrophilia may be associated with infections, drug side effects, tissue necrosis, inflammatory or metabolic disorders and hematopoietic neoplasms (CML, CNL). Neutrophilia is frequently associated lung and GI malignancies (Table 4-2).

#### Neutropenia

**Neutropenia** refers to an absolute neutrophil count (total leukocyte count per microliter  $\times$  percent of neutrophils) that is less than two standard deviations below the normal mean of the population (Table 4-3).

Causes of neutropenia:

1. Reduced production
2. Accelerated removal or utilization of circulating neutrophils

**TABLE  
4-2****Reactive neutrophilia vs chronic myelogenous leukemia (CML)**

	Reactive	CML
WBC	Usually <30K/ $\mu$ l	usually >30K/ $\mu$ l
Toxic appearance	present	absent
Left shift	present(myelocytes)	present (blasts)
Basophilia	absent	present
Nuc RBC	absent	present
Splenomegaly	absent	present
Uric acid	normal	increased
LAP*	increased	low
Cytogenetics/molecular studies	normal	t(9;22)

\*LAP: leukocyte Alkaline Phosphatase present in secondary granules of the maturing neutrophils from myelocytes to neutrophils.

**TABLE  
4-3****Diagnostic criteria of neutropenia**

Age group	Absolute neutrophil count
Infant	< 2500/ $\mu$ l ( $2.5 \times 10^9$ /L)
Adult	< 1500/ $\mu$ l ( $1.5 \times 10^9$ /L)

3. **Infection** is the most common cause of neutropenia **in neonates and infants**.
4. **T-cell large granular lymphocytic leukemia** patients are commonly present with neutropenia.
5. **Felty syndrome** characterized by rheumatoid arthritis, splenomegaly and neutropenia.
6. Cyclic neutropenia mutations in the neutrophil elastase ELA2 gene result in cyclical myeloid aplasia, often with rebound neutrophilia.
7. Kostmann syndrome (myeloid aplasia/hypoplasia, autosomal recessive)
8. Shwachman-Diamond syndrome (autosomal recessive)
9. Chédiak-Higashi syndrome (autosomal recessive)
10. Myelokathexis (autosomal dominant)
11. Fanconi anemia (autosomal recessive)
12. Dyskeratosis congenita (progress to aplasia, X-linked).

## Functional Defects of Neutrophils

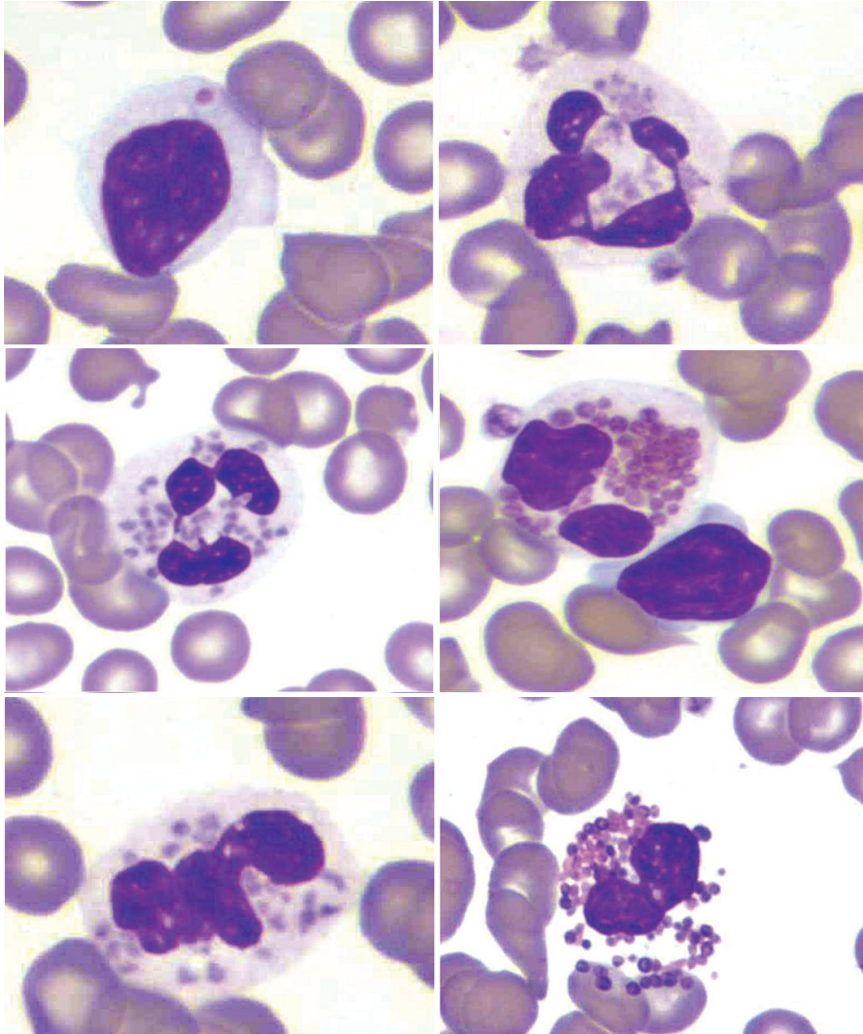
### *Degranulation Abnormalities*

1. **Chédiak-Higashi syndrome** is an autosomal recessive disorder with a defect in a lysosomal transport protein that affects the function of secretory



granules in various cells. Defects in the neutrophil chemotaxis, degranulation, and bactericidal activity result in increased susceptibility to infection that begins in infancy. This syndrome is also associated with albinism, neutropenia, platelet storage pool disorder, natural killer (NK) cell abnormalities, and peripheral neuropathies (Fig. 4-1).

2. Specific granule deficiency is an autosomal recessive disorder resulting in a functional loss of myeloid transcription factor.



**Fig. 4-1: Chediak-Higashi syndrome.** A large eosinophilic granule is seen in the cytoplasm of a lymphocyte (top left). Numerous large eosinophilic granules are seen in the cytoplasm of neutrophils (Peripheral blood smear) (Courtesy: Dr. Neil Harris, Department of Pathology, Immunology & Laboratory Medicine, University of Florida, College of Medicine, USA).



**Adhesion Abnormalities**

Leukocyte adhesion deficiency-1 and 2 is an autosomal recessive disorder resulting in neutrophils lacking surface adhesion molecules necessary for cell migration. It is characterized by neutrophilia and recurrent bacterial infections associated with lack of pus formation.

**Disorders of Decreased Cell Migration Response**

1. Defects in the generation of chemotactic signals
2. Intrinsic defects of the neutrophil (e.g. adhesion deficiency, Chédiak-Higashi syndrome, specific granule deficiency, neutrophil actin dysfunction, neonatal neutrophils).
3. Direct inhibition of neutrophil mobility, (e.g. drugs)
4. Immune complexes, bind to Fc receptors on the neutrophils (autoimmune diseases, e.g. Rheumatoid arthritis, SLE, and other inflammatory states)
5. Hyperimmunoglobulin E syndrome (autosomal dominant)

**Disorders of Microbicidal Activity**

The destruction of phagocytosed microbes is most effectively performed by an  $O_2$ -dependent process involving the reduction of oxygen (oxidative burst), and the addition of myeloperoxidase (MPO).

1. Chronic granulomatous disease (65% X-linked, 35% autosomal recessive) characterized by a deficiency of NADPH oxidase, which is needed for the reduction of  $O_2$  to  $H_2O_2$  (the first steps of the “oxidative burst”). The screening test (NBT test) will be negative (no color) due to the absence of NADPH oxidase.
2. Myeloperoxidase deficiency (autosomal recessive) characterized by the inability to convert  $H_2O_2$  (hydrogen peroxide) to  $HOCl$  (bleach) due to the lack of myeloperoxidase. The screening test (NBT test) will be positive (blue color) because the conversion of  $O_2$  to  $H_2O_2$  (by NADPH oxidase) is still intact.
3. Rac2 deficiency (autosomal recessive).
4. Deficiencies of glutathione reductase and glutathione synthetase.
5. Chédiak-Higashi syndrome (autosomal recessive).

**Hereditary Conditions Associated with Abnormal Neutrophil Morphology (Table 4-4)****Pelger-Huët Anomaly**

Pelger-Huët anomaly (autosomal recessive inheritance) is a benign disorder, with normal neutrophil function. This disorder is characterized by an abnormal

**TABLE  
4-4****Hereditary granulocytic disorders with abnormal morphology**

Pelger-Huet anomaly (autosomal dominant)	Normal neutrophil function, bilobed or non-segmented neutrophils, cytoplasm normal. Mutations in lamin $\beta$ -receptor
May-Hegglin anomaly (autosomal dominant)	Giant platelets, thrombocytopenia and large blue cytoplasmic inclusions resembling giant Döhle bodies present in neutrophils, eosinophils, basophils and monocytes. The presence of cytoplasmic inclusions and giant platelets are needed to make the diagnosis
Chédiak-Higashi syndrome (autosomal recessive)	Neutropenia, thrombocytopenia and function defect. All granulated cells including lymphocytes and NK cells are affected. Giant cytoplasmic granules represent fused lysosomes
Alder-Reilly anomaly (autosomal recessive)	Associated with genetic mucopolysaccharide disorders. Intense azurophilic granulation of neutrophil cytoplasm
Specific granule deficiency (autosomal recessive)	Recurrent infection, absent secondary granules
Myelokathexis (autosomal dominant or sporadic)	Neutrophils show hypersegmentation, pyknotic nuclei and cytoplasmic vacuoles
Hereditary hypersegmentation of neutrophils (autosomal dominant)	Normal function, increased nuclear lobes
Hereditary giant neutrophils (autosomal dominant)	Normal function, large neutrophils with increased number of lobes. Other cell lines are normal

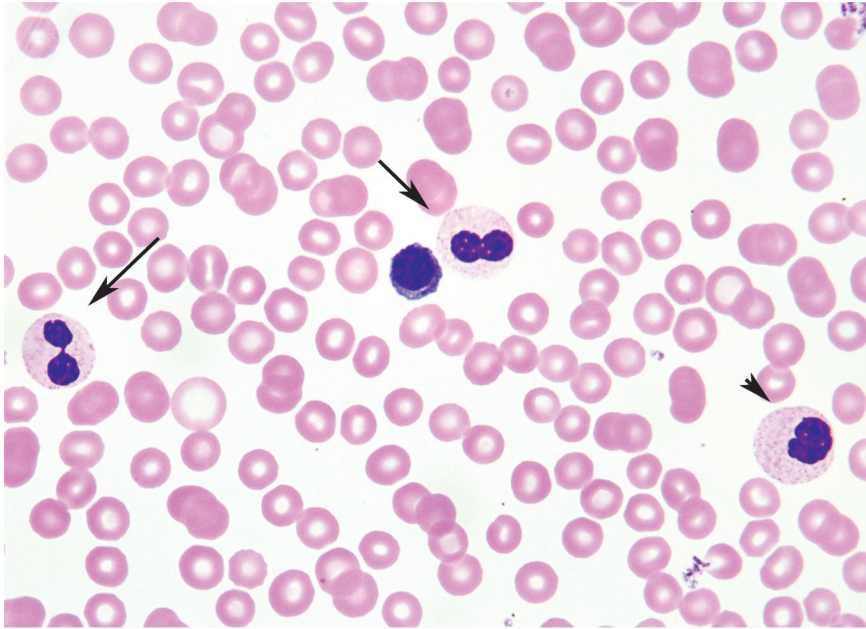
bi-lobed or mononucleated neutrophil morphology. The bi-lobed neutrophil has historically been referred to as “eyeglass-like” (“pince-nez” in French) appearance (Figs 4-2A and B).

### ***May-Hegglin Anomaly***

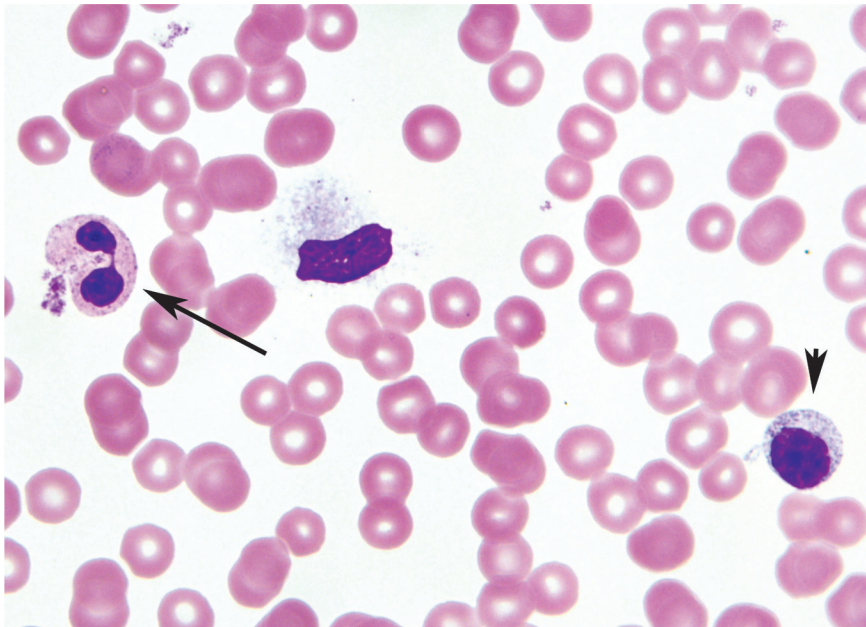
May-Hegglin anomaly (autosomal dominant inheritance) is due to a mutation in the protein-encoding sequence of MYH9. There is no effect on the normal function of neutrophils, however it does produce three characteristic features:

1. Döhle bodies in the cytoplasm of the neutrophils
2. Giant platelets
3. Thrombocytopenia.

A point mutation the MYH9 gene, results in the abnormal assembly of the non-muscle myosin heavy-chain. In addition to causing the formation of



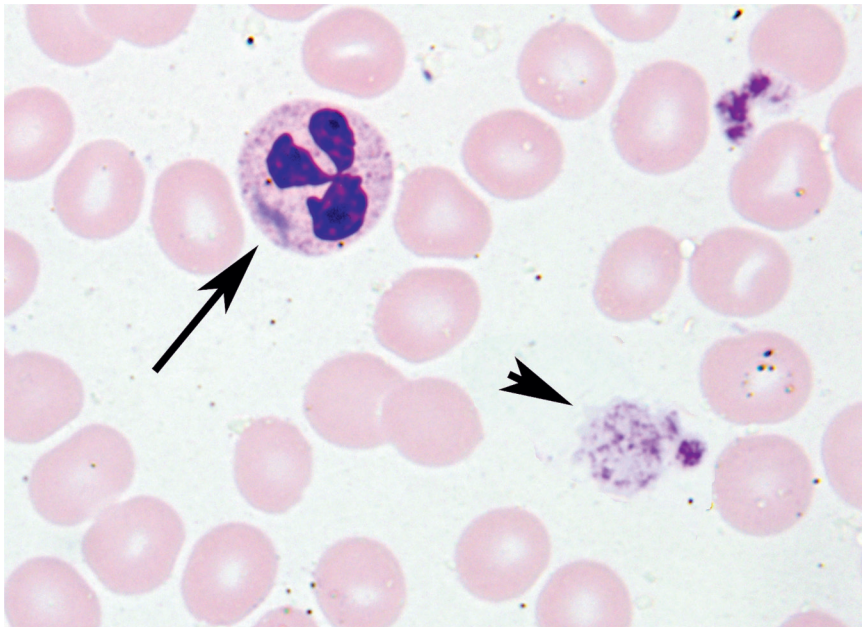
**Fig. 4-2A: Pelger-Huët anomaly.** Heterozygotes usually show bi-lobed or dumbbell-shaped neutrophil nuclei (arrow) (Peripheral blood smear).



**Fig. 4-2B: Pelger-Huët anomaly.** In the homozygotes usually show mono-lobed neutrophil nuclei (arrowhead) (Peripheral blood smear).

giant platelets and thrombocytopenia, this mutation also causes the precipitation of RNA from the rough endoplasmic reticulum, resulting in the formation of **Döhle bodies**.

Döhle bodies are light blue-gray colored inclusions, measuring 1-3  $\mu\text{m}$ , typically seen in the cytoplasm of neutrophils. These inclusions represent denatured aggregates of ribosomes (RNA). Döhle bodies are not specific for the May-Hegglin anomaly, and may be seen in cases of “toxic” neutrophil activation. However, in cases of May-Hegglin anomaly, the Döhle bodies inclusions are seen not only in neutrophils, but also in monocytes and lymphocytes (Fig. 4-3).

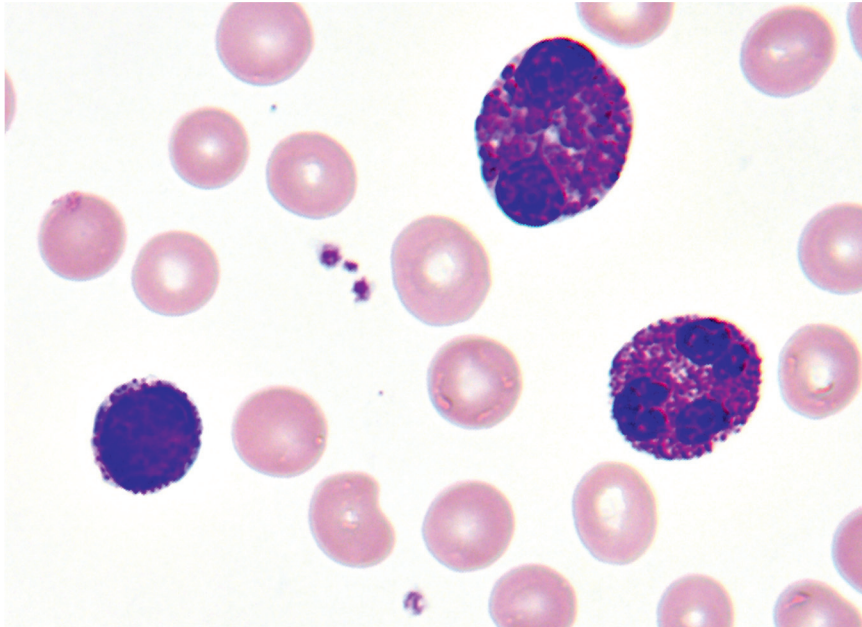


**Fig. 4-3: May-Hegglin anomaly.** Characteristic features of thrombocytopenia, giant platelet (arrowhead), and elliptical inclusions in neutrophil cytoplasm (arrow) (Peripheral blood smear).

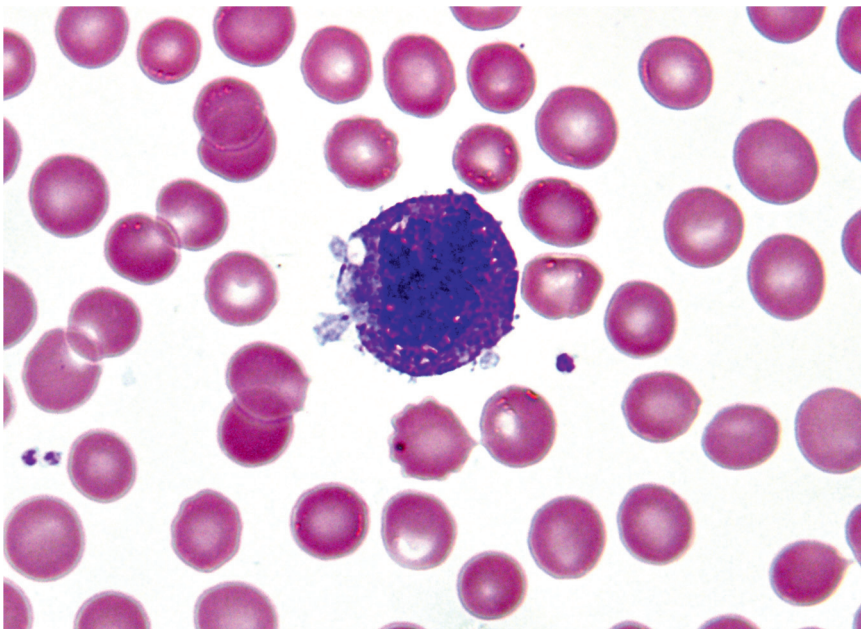
### ***Alder-Reilly Anomaly***

Alder-Reilly anomaly (autosomal recessive inheritance) is a rare disorder, typically associated with the group of mucopolysaccharidoses (MPS), and is due to a deficiency of lysosomal enzymes necessary to break down mucopolysaccharides. The accumulation of these substances in lysosomes, results in dense azurophilic granules seen in all leukocytes (neutrophils, monocytes, and lymphocytes), however the function of the leukocytes is not affected (Figs 4-4A and B).





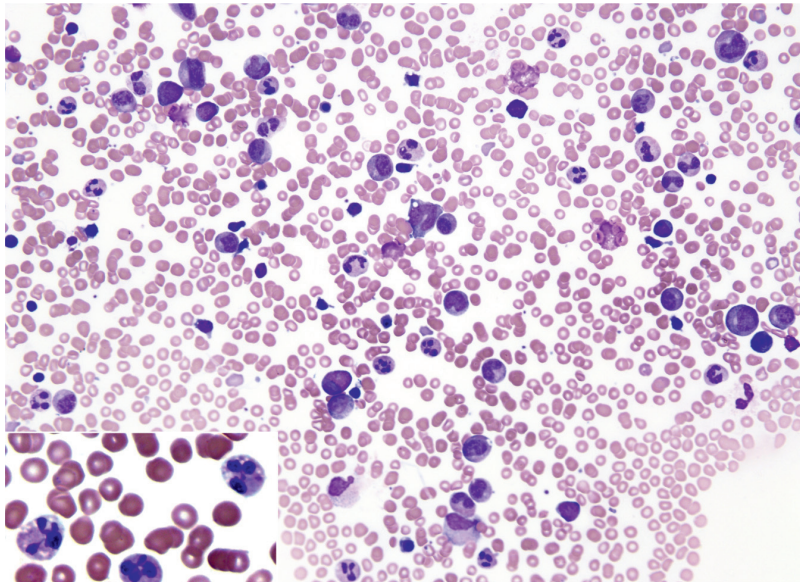
**Fig. 4-4A: Alder-Reilly anomaly.** Dense azurophilic granules present in neutrophils and lymphocytes (Peripheral blood smear).



**Fig. 4-4B: Alder-Reilly anomaly.** Dense azurophilic granules present in monocytes (Peripheral blood smear).

### **Myelokathexis**

Myelokathexis is a rare autosomal dominant disorder characterized by severe neutropenia, lymphocytopenia and growth retardation. The total white cell counts are often less than  $1000/\mu\text{l}$  ( $1.0 \times 10^9/\text{L}$ ). Myelokathexis is also part of WHIM syndrome (characterized by **W**arts, **H**ypogammaglobulinemia, **I**nfections, and **M**yelokathexis). The bone marrow usually shows abundant precursors and developing neutrophils. Neutrophils in the marrow and the peripheral blood show hypersegmentation with pyknotic nuclei and cytoplasmic vacuoles. Neutropenia may be partially corrected by G-CSF or GM-CSF treatment. Myelokathexis may progress to the myelodysplastic syndrome (Fig. 4-5).



**Fig. 4-5: Myelokathexis.** Abundant precursors and developing neutrophils. Neutrophils show hypersegmentation with pyknotic nuclei, degenerative changes, and cytoplasmic vacuoles (inset) (Bone marrow aspirate) (Courtesy: Dr Lei Shao, Department of Pathology, Children Mercy Hospital, Kansas City, MO, USA).

### **Eosinophilia**

**Eosinophilia** refers to an absolute eosinophil count of  $>500/\mu\text{l}$  ( $0.5 \times 10^9/\text{L}$ ).

Eosinophil production and function are profoundly influenced by interleukin-5 (IL-5).

An absolute eosinophil count of  $>500/\mu\text{l}$  commonly associated with:

1. Allergy
2. Parasitic infections



3. Dermatitis
4. Loeffler syndrome
5. T-cell and Hodgkin lymphoma
6. Langerhans cell histiocytosis
7. Interleukins IL1, IL3 and IL5 (IL5 is the most common one).
8. Chronic myelogenous leukemia, chronic eosinophilic leukemia, systemic mast cell disease, acute leukemia, and carcinoma.
9. Myeloid and lymphoid neoplasms with rearrangement of PDGFRA, PDGFRB and FGFR1.

Rearrangements of PDGFRA, PDGFRB and FGFR1 result in an aberrant tyrosine kinase. It is important to identify these rearrangements, because of the therapeutic option of tyrosine kinase inhibitors.

## Basophilia

**Basophilia** refers to an absolute basophil count of  $>200/\mu\text{l}$  ( $0.2 \times 10^9/\text{L}$ ).

Causes of basophilia:

1. Allergy
2. Inflammation
3. Endocrinopathy
4. Infection
5. Iron deficiency
6. Exposure to ionizing radiation
7. Neoplasia (CML).

Basophils may be closely related to mast cells, but the association is controversial. Basophils circulate as mature cells and do not reside in tissues, but can be recruited into tissues at sites of immunologic or inflammatory responses. By contrast, mast cells typically are derived from blood precursors that lack many of the characteristic features of the mature cells and complete their maturation in the tissues. The mature mast cells can reside in tissues for long periods (Table 4-5).

## Monocytosis

**Monocytosis** refers to an absolute monocyte count of  $>1000/\mu\text{l}$  ( $> 1 \times 10^9/\text{L}$ ).

Monocytes are normally accounted for 2-9% of the total WBC count and the absolute count is normally 100-900/ $\mu\text{l}$ .

**TABLE  
4-5****Comparison of basal cells and mast cells**

	<b>Basophil</b>	<b>Mast cell</b>
Size	15-30 mm	10-15 mm
Nucleus	Round	Segmented
Cytoplasm	Abundant	Moderate
Granules	Evenly distributed	Random distribution
Cytochemistry	Chloracetate esterase positive	Peroxidase positive
Location	Bone marrow	Blood or bone marrow
Mediated by IL3, IL5 and GM-CSF	Yes	Yes

***Causes of Reactive Monocytosis***

1. Infection
2. Inflammatory condition
3. ITP
4. Post-splenectomy
5. Neoplasm (lymphoma, myeloma and cytokine-producing carcinoma).

***Causes of Neoplastic Monocytosis***

1. MDS/MPD
2. AML
3. CML
4. Pediatric MDS/MPD with monosomy 7.

**Benign Lymphocytosis**

Lymphocytosis is defined as an absolute lymphocyte count  $>4000/\mu\text{l}$  ( $>4 \times 10^9/\text{L}$ ).

***Causes of Benign Lymphocytosis***

1. Infectious mononucleosis (EBV infection).
2. Whooping cough (***Bordetella pertussis***): Associated with neutrophilia, mature-appearing lymphocytosis, and atypical lymphocytes.
3. Drug reaction (anticonvulsant therapy—phenytoin).
4. Persistent polyclonal B-cell lymphocytosis: Unknown cause, may associate with smoking.

5. Other infections:
  - CMV
  - Adenovirus
  - HIV
  - HHV-6
  - Hepatitis A, B, and C
  - Rubella
  - Toxoplasmosis.

### ***Diagnosis and Differential Diagnosis***

1. Atypical lymphocytosis and positive initial screening test for heterophil antibody and serology test for EBV antibodies (Table 4-6).
2. CMV infection may contain Reed-Sternberg like cells (CD15+, CD20-, CD30-, CD45-). Positive CMV immunohistochemical staining and viral inclusion is helpful to distinguish EBV and CMV infection.

## **Lymphocytopenia**

Lymphocytopenia is defined as a total lymphocyte count less than  $<1000/\mu\text{l}$  ( $1.0 \times 10^9/\text{L}$ ) in adults or less than  $<2000/\mu\text{l}$  ( $2.0 \times 10^9/\text{L}$ ) in pediatric patients (less than 16 years old).

### ***Causes of Lymphocytopenia***

1. Congenital immunodeficiency disorders:
  - Severe combined immunodeficiency disease (SCID)
  - DiGeorge syndrome
  - Wiskott-Aldrich syndrome
2. Infectious diseases

**TABLE  
4-6**

Serologic profile in infectious mononucleosis (EBV infection)

Antibody	Acute phase	Chronic phase
Heterophile	Positive	Negative
Viral capsid antigen (VCA) – IgM	Positive	Negative
Viral capsid antigen (VCA) – IgG	Positive	Positive
EBV nuclear antigen – IgG	Negative	Positive
EBV early antigen (EA) -IgG	Negative	Positive

3. Autoimmune disorders
  - Rheumatoid arthritis (RA)
  - Systemic lupus erythematosus (SLE)
  - Myasthenia gravis
4. Others:
  - Burns
  - Sarcoidosis
  - Malignancy
5. Iatrogenic and idiopathic.

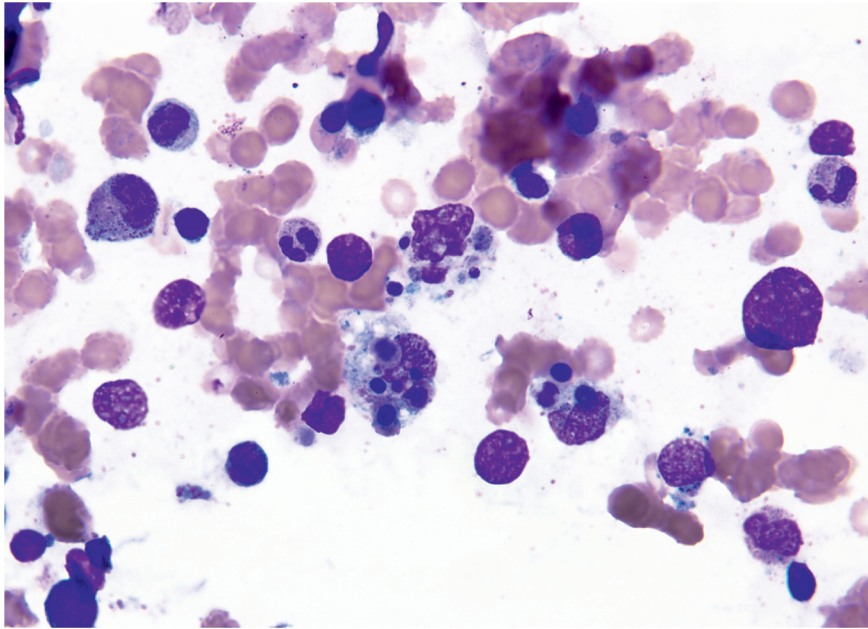
## Hemophagocytic Lymphohistiocytosis (HLH)

1. **Primary HLH:** Autosomal recessive familial hemophagocytic lymphohistiocytosis.
2. **Acquired HLH** is caused by strong immunological activation of the immune system (i.e. infection).

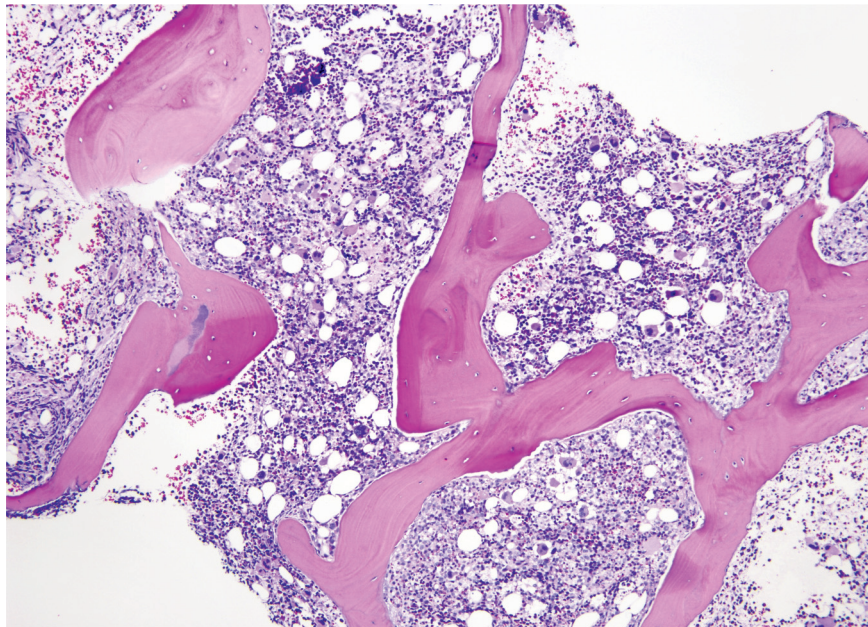
In general, clinical features and outcomes are the same for both primary and acquired HLH. Functional defects of natural killer (NK) cells and cytotoxic T-cells result in an inappropriate activation of T-cells/macrophages, multiorgan dysfunction, and death.

Early clinical signs included fever, hepatomegaly, splenomegaly, neurologic symptoms, rash, and lymphadenopathy. Important clinical clues are an acutely ill patient with unexplained fever, rash, or neurologic symptoms. A patient with immune deficiency, recurrent spontaneous abortions, or family history of HLH should prompt full evaluation for HLH.

Finding hemophagocytosis is highly suggestive of HLH, but is neither necessary nor sufficient to make the diagnosis (Table 4-7). Hemophagocytosis may be modest in the early phase or rare when the bone marrow progresses to aplastic with few macrophages available to engage in hemophagocytosis. Biopsies of bone marrow, spleen or lymph node fail to demonstrate hemophagocytosis in approximately one-third of patients. Repeat marrow aspirates and biopsies, as well as lymph node or liver biopsies, may be helpful (Figs 4-6A to C). Cerebrospinal fluid should be tested in patients with signs of CNS abnormalities; pleocytosis and hyperproteinemia support HLH with CNS involvement.

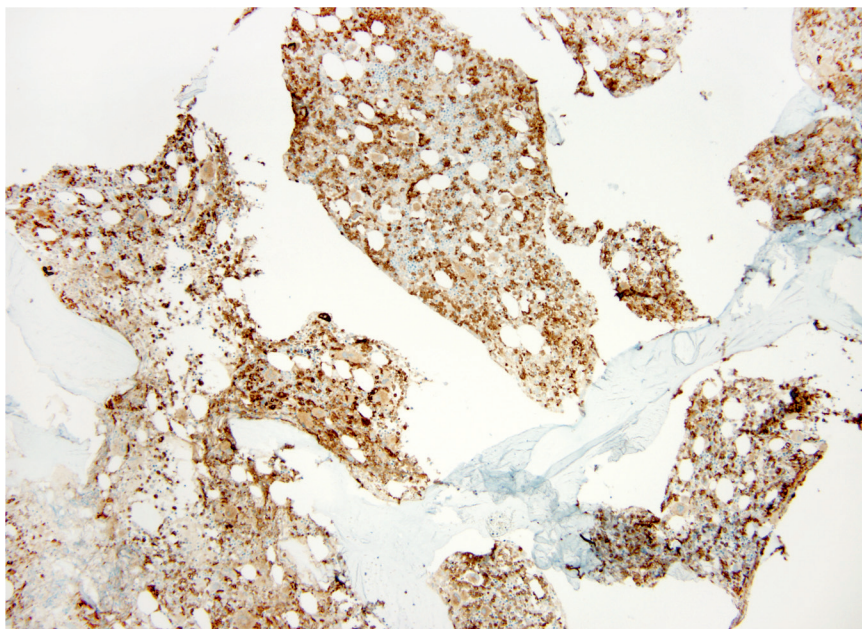


**Fig. 4-6A: Hemophagocytic lymphohistiocytosis.** Hemophagocytosis of multiple cells by macrophages (Bone marrow aspirate).



**Fig. 4-6B: Hemophagocytic lymphohistiocytosis.** Bone marrow showing reduced hematopoiesis and fibrosis (Bone marrow section).





**Fig. 4-6C: Hemophagocytic lymphohistiocytosis.** Immunohistochemical stain showing a marked increase of CD68 positive macrophages (Bone marrow section).

**TABLE  
4-7**

Diagnosis of HLH can be established if either of the two criteria is fulfilled

- |   |  |
|---|--|
| 1 | A molecular diagnosis consistent with HLH*   |
| 2 | Five out of eight diagnostic criteria are fulfilled <ol style="list-style-type: none"> <li>1. Fever</li> <li>2. Splenomegaly</li> <li>3. Cytopenias (affecting <math>\geq 2</math> of 3 lineages in the peripheral blood)</li> <li>4. Hypertriglyceridemia and/or hypofibrinogenemia</li> <li>5. Hemophagocytosis in bone marrow or spleen or lymph node, with no evidence of malignancy</li> <li>6. Low or absent NK-cell activity</li> <li>7. Ferritin <math>\geq 500</math> mg/L</li> <li>8. Elevated level of soluble IL-2 receptor (CD25) <math>\geq 2,400</math> U/ml</li> </ol> |

\* including:

Perforin (PRF1) gene mutation (10q21-22)  
 UNC13D(17q25) gene mutation  
 STX11 (6q24) gene defect

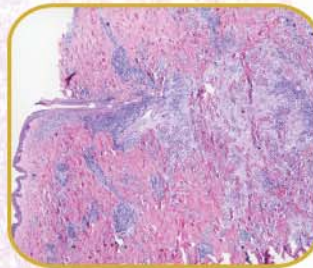




CHAPTER

5

# Normal Lymph Node, Thymus and Lymphoid Hyperplasia



## *Normal Lymph Node—Function and Structure*

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### Major Functions

1. Lymphopoiesis
2. Filtration
3. Processing antigens.

### Structure

1. **Cortical region (Cortex):** The outer region of the lymph node, just underneath the capsule of the lymph node. This area contains both primary follicles (inactive B-cell regions) and secondary follicles (activated follicles with germinal centers). Activated germinal centers contain predominantly B-cells (centrocytes and centroblasts), tingible body macrophages, follicular dendritic cells, and few T-cells. The follicles are surrounded by a rim of B-cells, called the mantle zone. The outer-most, pale staining region of the mantle zone is referred to as the marginal zone, however this is usually not visually evident on lymph node specimen.

Immunohistochemical staining patterns:

- a. Germinal center: Positive for CD20, CD79a, CD10, BCL6, and IgM (late shift to IgG or rare IgA). **IgD** and **BCL2** are negative.
  - b. Mantle zone: Positive for CD20, CD5, **IgD**, and **IgM**.
  - c. Follicular dendritic cells: Positive for CD21 and CD23.
2. **Paracortical region (Paracortex):** The area between the follicles, which extends deeper into the node, and consists predominantly T-cells, interdigitating dendritic cells, and small blood vessels.

Immunohistochemical staining pattern:

- a. T-cells: Positive for CD2, CD3, CD5, CD7, CD43, BCL2, and either CD4 or CD8. The normal CD4 to CD8 ratio is 3:1.
  - b. Interdigitating dendritic cells: Positive for HLA-DR.
3. **Medullary region (Medulla):** The area in the hilar region of the lymph node. Medullary “cords” are the cellular areas in between the sinuses (which are prominent in the medullary region). This is the main site of differentiation and proliferation of plasma cells.

Immunohistochemical staining patterns: Plasma cells: positive for CD138, heavy chains (IgM, IgG, IgA), and light chains (kappa, lambda). The normal kappa to lambda ratio is 2:1.

4. **Sinuses:** The lymphatic sinuses run throughout all regions of the lymph node, however they are most easily visualized in the medullary region, and in the subcapsular region of the cortex. The subcapsular sinuses have an

endothelial lining, however, as they traverse through the node, they lose this lining and acquire a variable amount of macrophages (histiocytes).

Immunohistochemical staining patterns:

- a. Macrophages: Positive for CD68
- b. Langerhans cells: positive for CD1a and S-100.

## *Normal Thymus—Function and Structure*

### Major Functions

1. Antigen-independent T-cell maturation (T-cell precursors from bone marrow to subcapsular cortex, mature naïve T-cells move to peripheral blood via cortex or medulla perivascular spaces).
2. Negative selection of self-reactive clones.
3. Positive selection of MHC-recognized clones.

### Structure

1. Cortex contains large stellate epithelial cells with prominent nucleoli, medium sized blastic T lymphocytes and macrophages.
2. Medulla contains small spindle epithelial cells, Hassall's corpuscles, mature T lymphocytes and dendritic cells.
3. Immunophenotype (Table 5-1).

**TABLE  
5-1**

**Comparison of T-cell phenotypes in the cortex and medulla of the thymus**

Cortex	Medulla
TdT + CD4+ and CD8+ (dual positive) CD1a+	TdT - CD4+ or CD8+ CD1a-

## *Predominant Patterns and Etiology of Reactive Lymph Node Hyperplasia*

### Follicular Hyperplasia

1. Reactive follicular hyperplasia (rule out follicular lymphoma Table 5-2)
2. Rheumatoid arthritis
3. HIV (early)
4. Castleman's disease
5. Toxoplasmosis

**TABLE  
5-2**

**Reactive follicular hyperplasia versus follicular lymphoma**

Reactive follicular hyperplasia	Follicular lymphoma
BCL2- in germinal center CD10+ in germinal center BCL6+ in germinal center MIB-1 high in germinal center (near 100% with a sharply demarcated margin) No light chain restriction t(14;18) absent	BCL2+ in germinal center CD10+/- in germinal center BCL6+/- in germinal center MIB-1 low in germinal center (10-50%)  Light chain restriction or both absent t(14;18) present

6. Syphilis
7. Bacterial infection (early)
8. Progressive transformation of germinal centers (Rule out follicular lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma).

### Paracortical Hyperplasia

1. Dermatopathic lymphadenopathy
2. Viral infection
3. Postvaccination lymphadenopathy
4. Drug-induced hypersensitivity reaction
5. Kikuchi disease
6. SLE
7. Draining region of suppurative inflammation or carcinoma.

### Medulla/Sinuses—Distended Sinuses

1. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)
2. Whipple's disease (some cases)
3. Reactive to foreign material.

### Lymph Nodes with Necrosis (High-Grade B-cell lymphoma should be Ruled Out First)

1. Cat-scratch disease (*Bartonella henselae*, PCR test).
2. Kikuchi-Fujimoto lymphadenitis (See Kikuchi disease).
3. Infarction (commonly seen after fine needle aspiration procedure).

### Lymph Node with Granuloma

1. Sarcoidosis
2. Mycobacterial infection

3. Fungal infections
4. Drugs
5. Hodgkin lymphoma.

### *Highlighted Topics*

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#### **Sinus Histiocytosis with Massive Lymphadenopathy (Rosai-Dorfman Disease)**

Sinus histiocytosis with massive lymphadenopathy is a polyclonal disorder of unknown etiology. The typical patient is a healthy young adult who presents with massive cervical lymphadenopathy with fever, hypergammaglobulinemia and an elevated sedimentation rate. The disease is usually self-limited. Rare deaths are attributed to immune dysregulation or visceral involvement. Unlike Langerhans cells, which are positive for S-100 and CD1a, these histiocytes in the sinus only express **S-100 but not CD1a**.

Extranodal Rosai-Dorfman disease is uncommon, but does occur. Rosai-Dorfman disease may involve breast, subcutaneous tissue or other extranodal sites (Figs 5-1A to F).

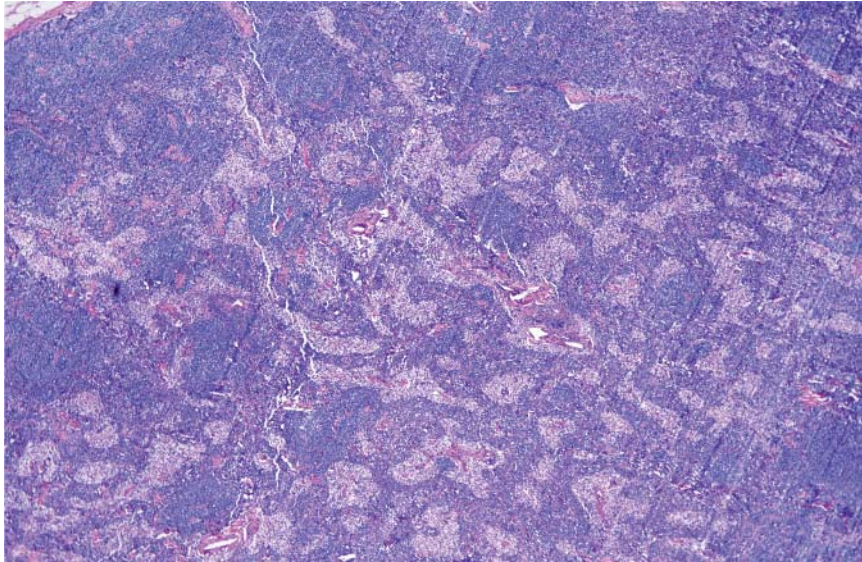
#### **Progressive Transformation of Germinal Centers**

Progressive transformation of germinal centers (PTGC) is a benign disorder of unknown etiology. It commonly presents as localized lymphadenopathy in a young adult. The histology often shows follicular hyperplasia, and a few large blue nodules which represent germinal centers that have been infiltrated and replaced by mantle zone B-cells. These large nodules are readily identifiable, as they are larger than the surrounding reactive follicles (Fig. 5-2). PTGC may be associated with nodular lymphocyte-predominant Hodgkin lymphoma, so a close examination must be made for the presence of “popcorn” or lymphocyte predominant cells (LP cells) in the closely packed nodular areas.

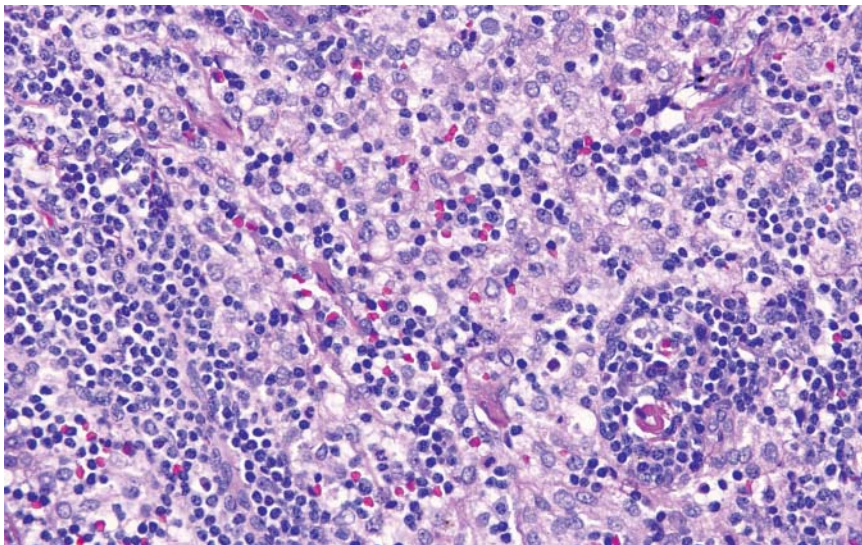
#### **Vascular Transformation of Sinuses**

Vascular transformation of sinuses (VTS) is a rare condition, which the sinus of lymph node is converted into a complex network of anastomosing, endothelial-lined channels. The nodular spindle cell variant is composed of interlacing fascicles alternating with vascular clefts. This variant can be confused with Kaposi’s sarcoma involving the lymph node. Nodular spindle cell variant is confined to the sinuses with sparing of the capsule and parenchyma.



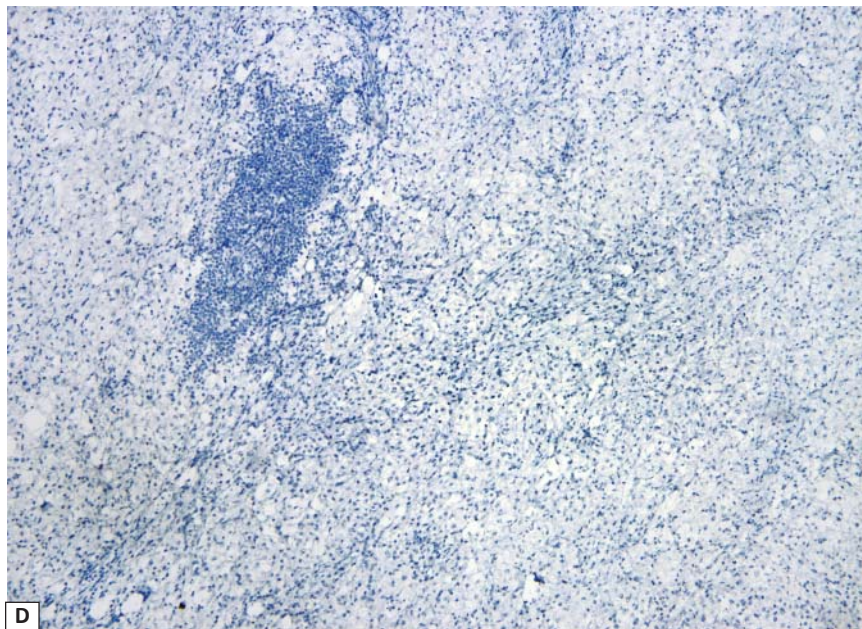
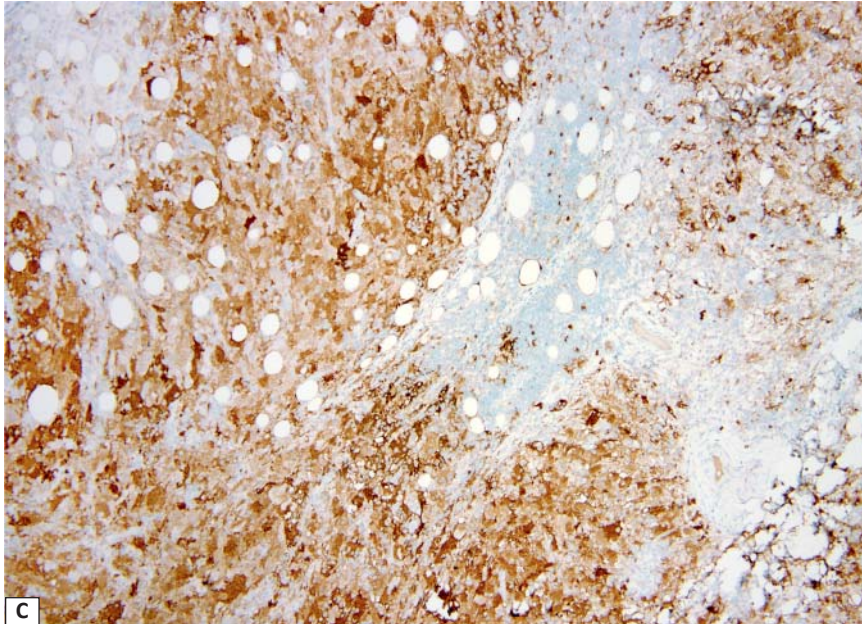


**Fig. 5-1A: Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease).** The lymph node sinuses are expanded by a proliferation of histiocytes, which can be appreciated on low power (Lymph node biopsy).



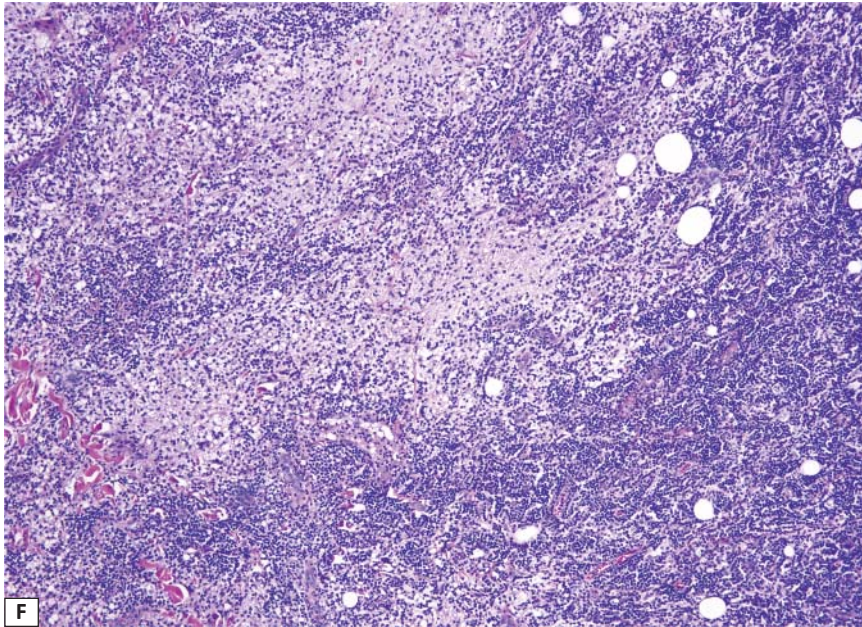
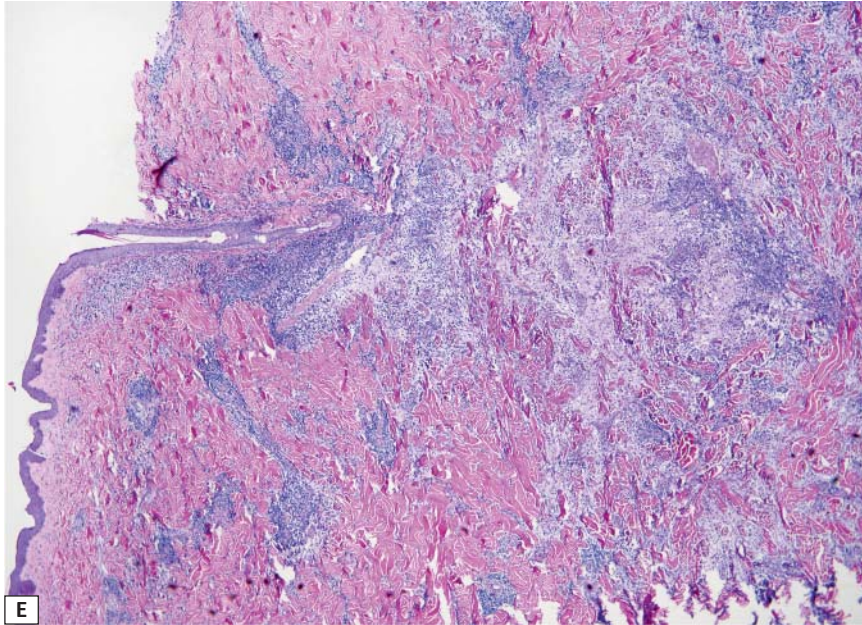
**Fig. 5-1B: Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease).** The histiocytic cytoplasm is pale and eosinophilic. The diagnostic finding is intact lymphocytes within macrophages (lymphophagocytosis, or emperipolesis—the penetration of a smaller cell into larger one). Because the lymphocytes are inside vacuoles, they are not degraded. Numerous plasma cells are also present. The pathologic macrophages are phagocytosing lymphocytes and plasma cells as well as erythrocytes (Lymph node biopsy).



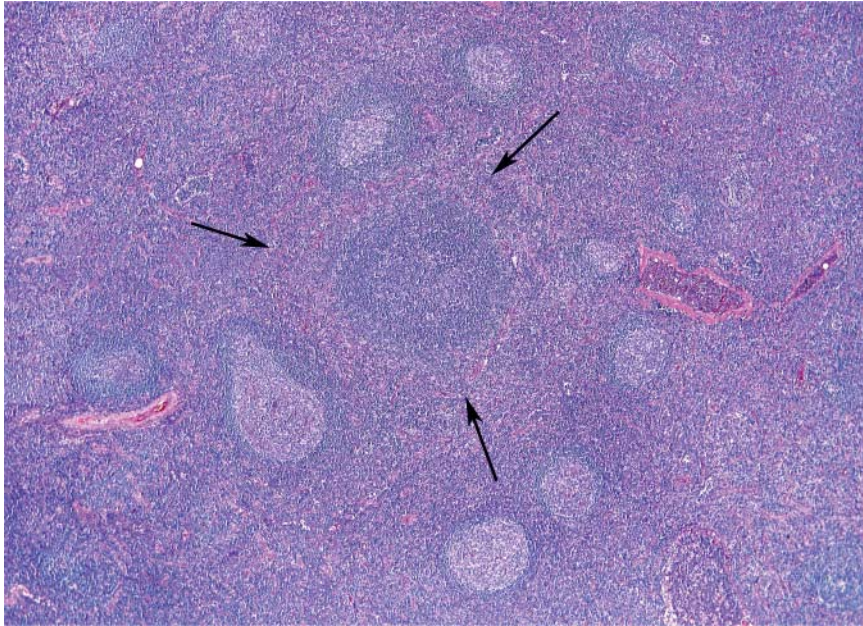


**Figs 5-1C and D: Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease).** In contrast to Langerhans cells, which are positive for both S-100 and CD1a, the histiocytes in Rosai-Dorfman disease are positive for S100 (C), but negative for CD1a (D) (Lymph node biopsy).





**Figs 5-1E and F: Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)** may also involve subcutaneous tissue (E) and breast tissue (F) (Skin and breast biopsies).



**Fig. 5-2: Progressive transformation of germinal centers (PTGC).** LA large blue nodule, typically larger than the surrounding reactive follicles, represents a germinal center infiltrated and replaced by mantle zone B-cells (arrows) (Lymph node biopsy)

### Bacillary Angiomatosis

*Bartonella* species are responsible for a wide variety of clinical syndromes including trench fever and cat scratch disease. Bacillary angiomatosis occurs almost exclusively in immunocompromised patients (especially HIV infection).

Trench fever is a self-limited, louse-borne relapsing febrile disease caused by *Bartonella quintana*.

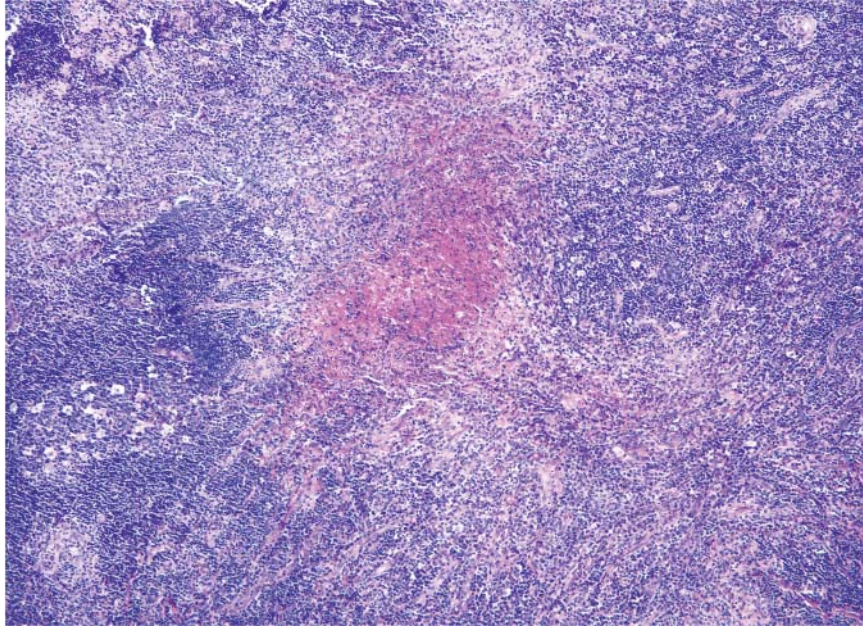
Cat-scratch disease is an acute infection of children and young adults caused by ***Bartonella henselae***. Bacillary angiomatosis, peliosis hepatis and retinitis are disseminated forms of the disease.

The lymph node shows multiple coalescent intranodal clusters of proliferating vessels with abundant amorphous or granular eosinophilic materials. Warthin-Starry stain reveals bacillary organisms (Fig. 5-3).

### Kaposi's Sarcoma of Lymph Node

Kaposi's sarcoma in the lymph node shows a proliferation of spindle cells with slit-like spaces containing red blood cells. In the early stage, the lesion involves subcapsular and trabecular sinuses. In the late stage, the lesion involves the entire lymph node.





**Fig. 5-3: Cat scratch disease.** Irregular shaped granuloma with central necrosis and vascular proliferations (Lymph node biopsy).

## Castleman's Disease

Castleman's disease is a rare, benign lymphoepithelial disease with the potential for development of Kaposi sarcoma, lymphoma and **follicular dendritic cell tumor**. This disease can occur at any age but young adults are common, and affect males and females equally. The most commonly site of involvement is the thoracic lymph nodes. There are two histologic variants: hyaline-vascular (80-90% of cases) and plasma cell variant (10-20% of cases).

1. **Hyaline-vascular variant** is characterized by atrophic lymphoid follicles with small, hyalinized germinal center and broad mantle zone arranged in concentric rings around the germinal center (onion skinning pattern). The germinal center contains an increased proportion of follicular dendritic cells, often penetrated by a blood vessel ("lollipop"). The interfollicular region contains vessels and occasionally cluster of plasma cells. Sheet of plasma cells are absent.
2. **Plasma cell variant** occurs in two forms: localized or multicentric and commonly associated with herpes virus (HHV-8) infection. The multicentric plasma cell variant of Castleman's disease may associate with POEMS syndrome (**P**olyneuropathy, **O**rganomegaly, **E**ndocrinopathy, **M**onoclonal gammopathy or **M**ultiple myeloma or **M** spike, and **S**kin changes).

In about 50% of cases, plasma cells are monoclonal and nearly always IgG $\lambda$  or IgA $\lambda$ .

Localized Castleman's disease is managed by surgical resection with excellent prognosis. The multicentric plasma cell variant carries a worse prognosis, and is usually treated with a combination of chemotherapy and steroids (Figs 5-4A to E).

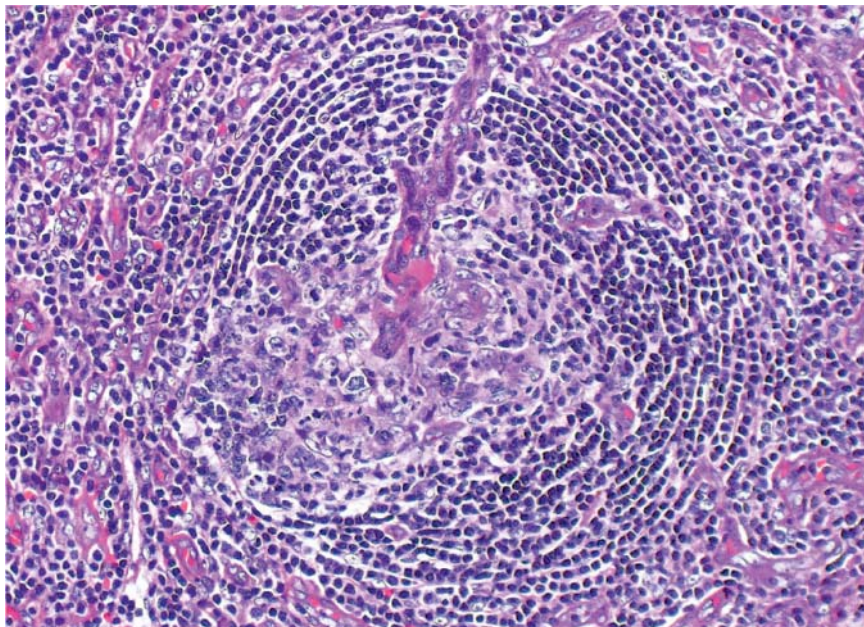
### **Dermatopathic Lymphadenopathy**

Dermatopathic lymphadenopathy is a reactive paracortical hyperplasia of lymph node characterized by increased number of interdigitating dendritic cells, Langerhans cells, and histiocytes with cytoplasmic melanin deposits and lipid vacuoles.

Dermatopathic lymphadenopathy is usually associated with skin disease. The most commonly involved sites are axillary and inguinal lymph nodes (Figs 5-5A-B).

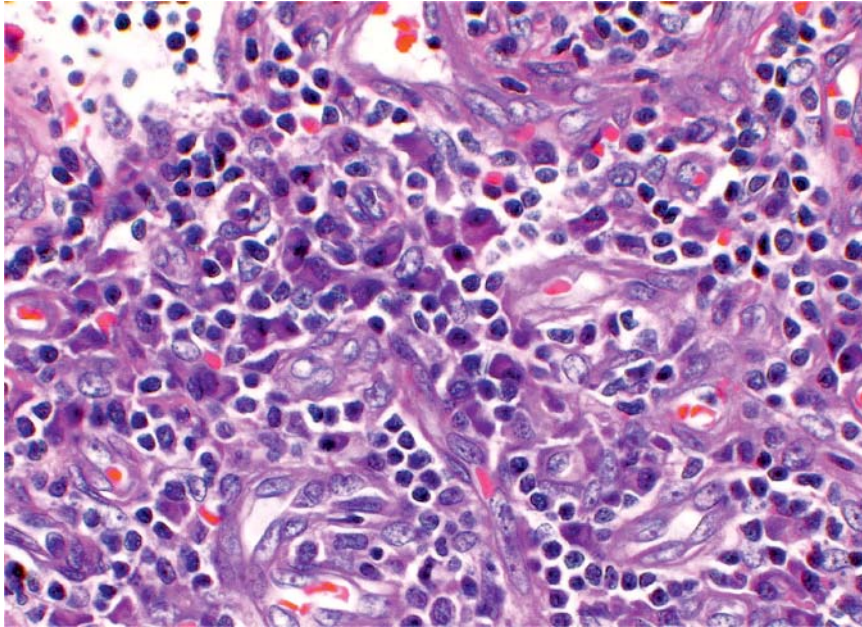
### **Kimura Lymphadenopathy (Kimura Disease)**

Kimura disease is a self-limiting non-neoplastic disorder prevalent in Asians. Kimura disease involves subcutaneous tissue and lymph nodes predominantly in

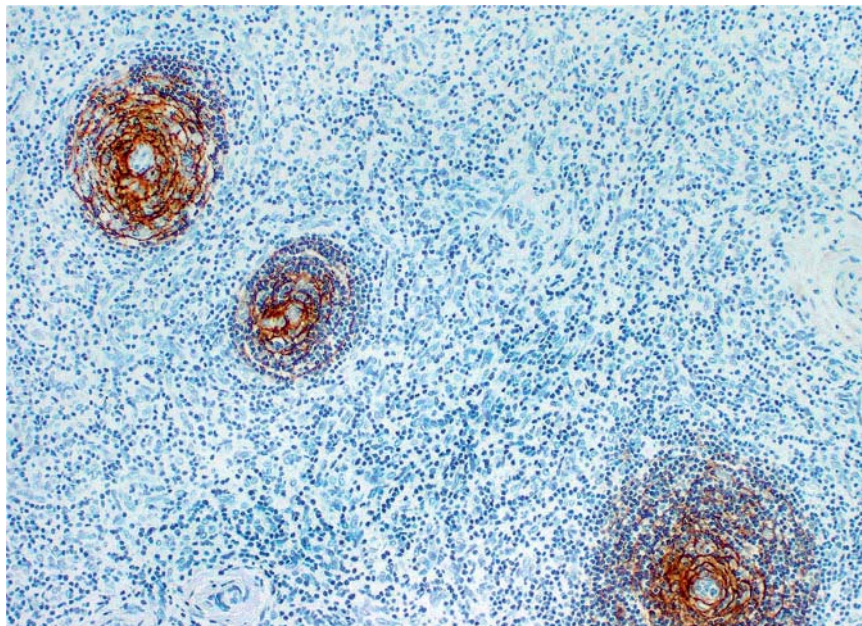


**Fig. 5-4A: Castleman's disease, hyaline-vascular variant.** The atrophic, sclerotic germinal center is penetrated by a blood vessel ("lollipop" appearance), surrounded by concentric rings of mantle zone B-cells ("onion-skin" appearance) (Lymph node biopsy).



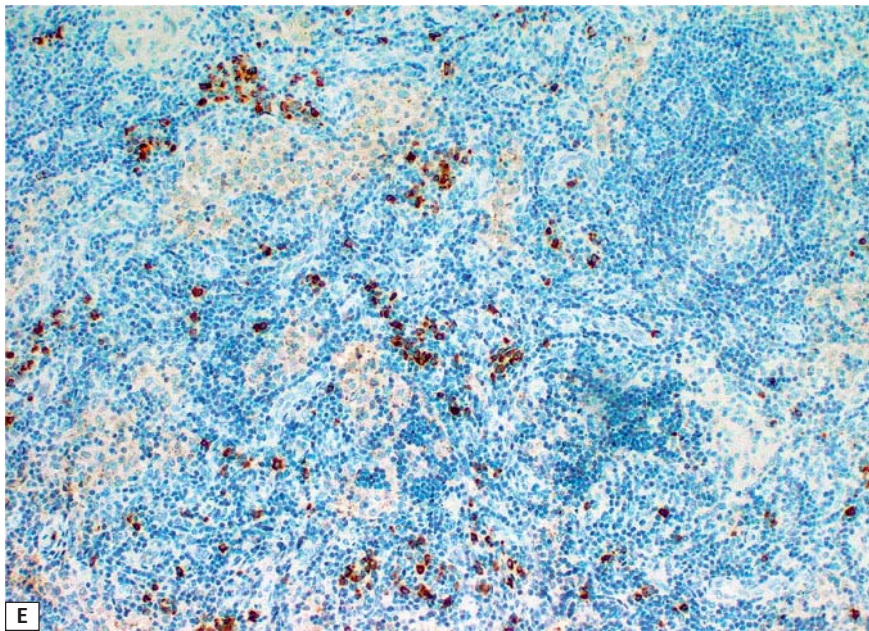
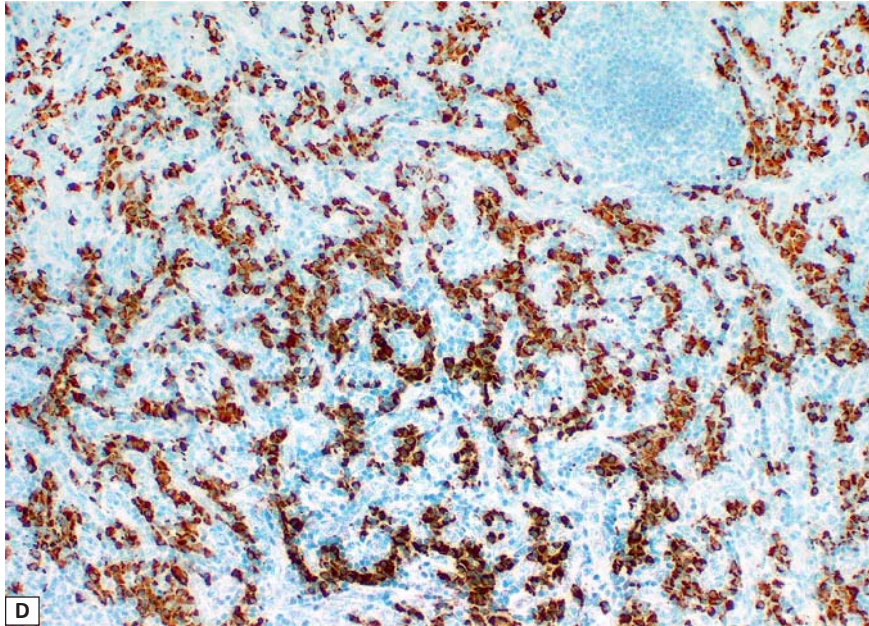


**Fig. 5-4B: Castleman's disease, hyaline-vascular variant.** In the interfollicular region, small clusters or aggregates of plasma cells can be seen, but sheets of plasma cell are absent (Lymph node biopsy).



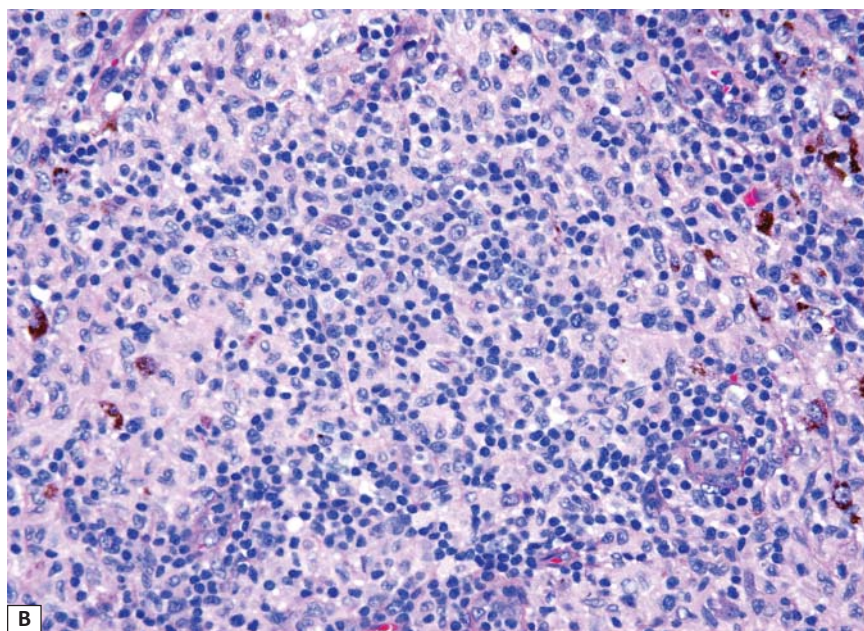
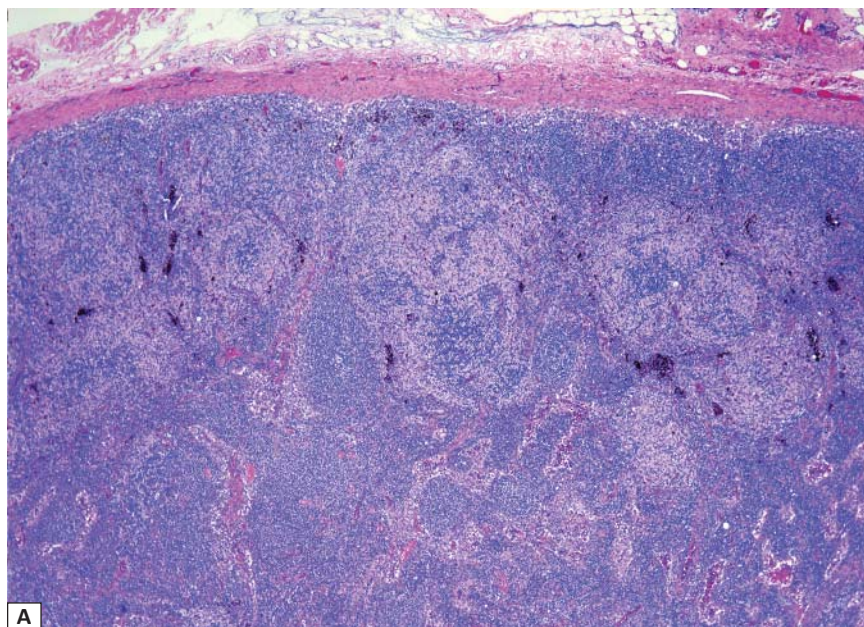
**Fig. 5-4C: Castleman's disease, hyaline-vascular variant.** CD21 stain showing increased follicular dendritic cells in the germinal center (Lymph node biopsy).





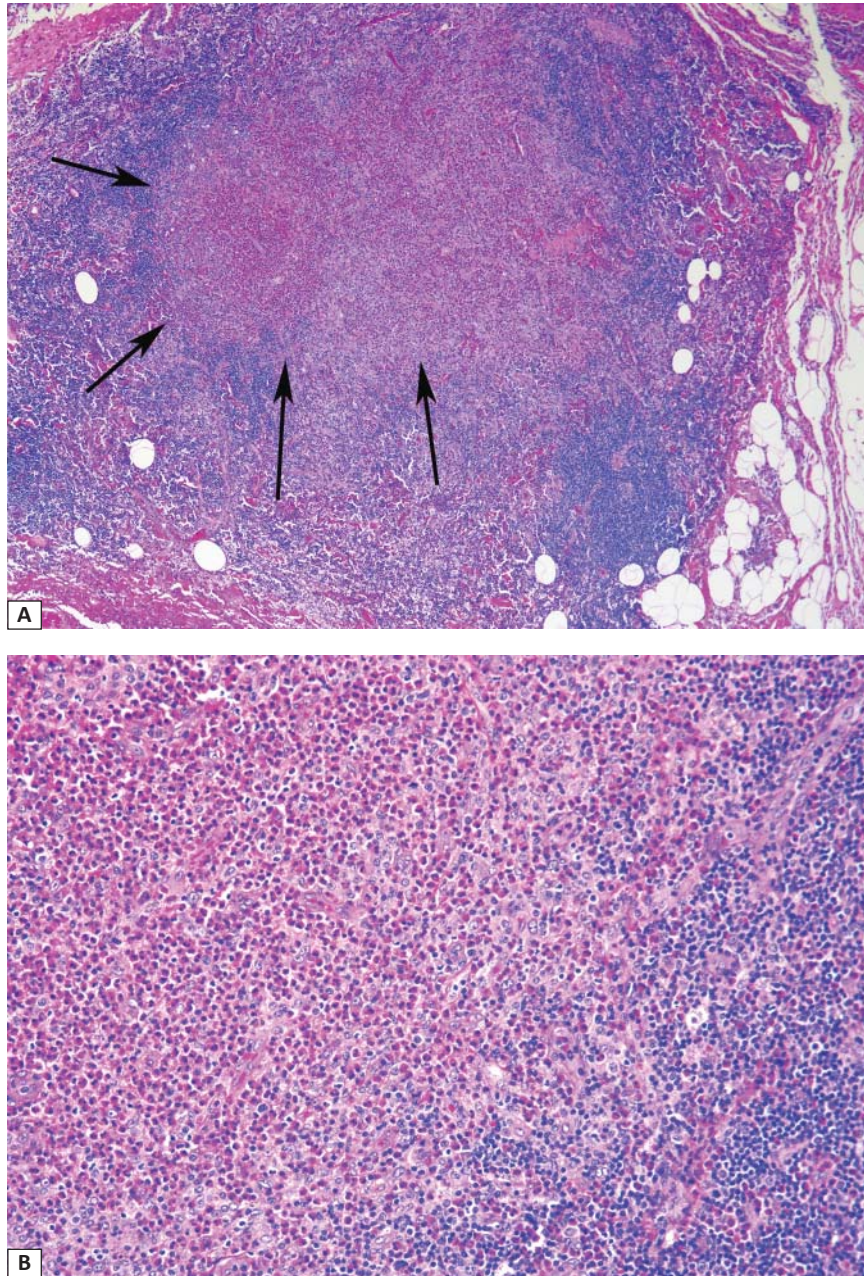
**Figs 5-4D and E: Castleman's disease, plasma cell variant.** Immunohistochemical stains highlight the numerous plasma cells in the interfollicular (paracortex) region. In this case, there is a monoclonal plasma cell population, staining strongly for lambda light chain (D), with few plasma cells staining for Kappa light chain (E).





**Figs 5-5A and B: Dermatopathic lymphadenopathy.** (A) The lymph node shows paracortical hyperplasia and (B) pigment containing histiocytes at high power (Inguinal lymph node biopsy from a patient with history of chronic skin lesion).



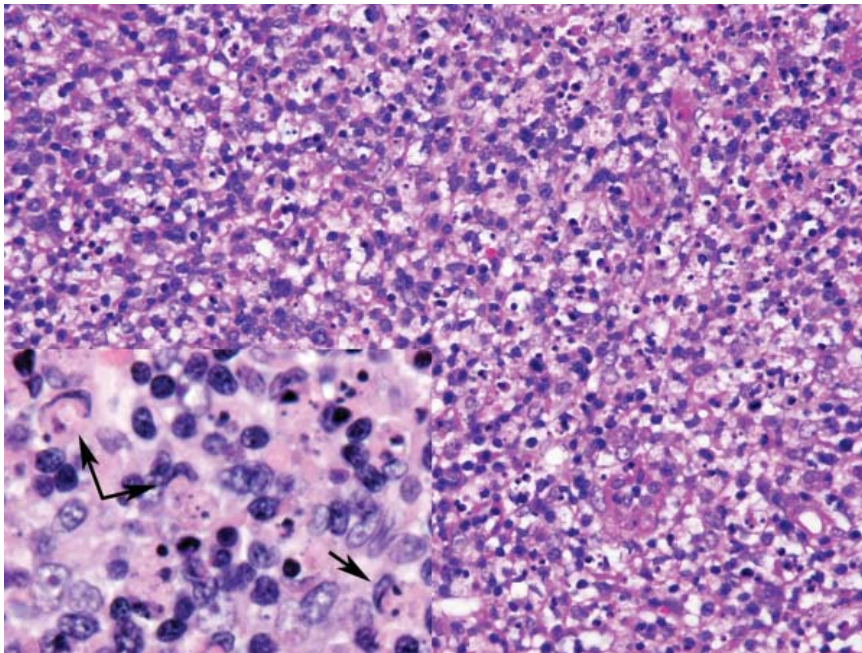


**Figs 5-6A and B: Kimura disease.** Lymph node shows eosinophilic microabscess (A and B). These eosinophilic microabscesses are frequently seen within follicles, subcapsular and paracortical areas. Follicular hyperplasia may present.

the head and neck region and is characterized by angiolymphoid proliferation and eosinophilia. Most patients are in their late 20 to 40 years old. Male to female ratio is 3 to 1 (Figs 5-6A and B).

### **Kikuchi Disease**

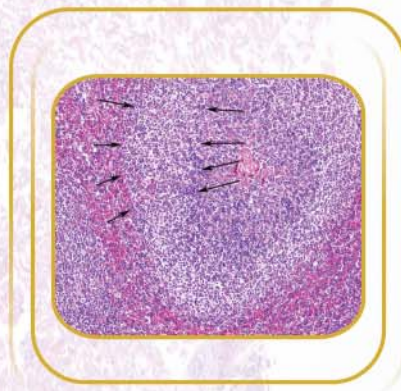
Kikuchi disease (also known as Kikuchi histiocytic necrotizing lymphadenitis, Kikuchi lymphadenitis, Kikuchi-Fujimoto disease, and subacute necrotizing lymphadenitis) is an unknown etiology and self-limited disease that primarily affects young Asian women. Patients typically present as unilateral, frequently painful, cervical lymph node enlargement. Hepatosplenomegaly or generalized lymphadenopathy may present. Lymph node biopsy shows a mixture of lymphocytes, plasmacytoid monocytes, crescentic histiocytes, histiocytes and areas of karyorrhexis conspicuously absent neutrophils. Histiocytes are positive for CD68+ and MPO+. CD8+ T-cells are abundant (Fig. 5-7).



**Fig. 5-7: Kikuchi disease:** In the area near the necrosis shows mixture of lymphocytes, plasmacytoid monocytes, crescentic histiocytes (inset), histiocytes, apoptotic and eosinophilic debris. Neutrophils are usually absent (Lymph node biopsy).



# Spleen





## *Normal Spleen Function and Structure*

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### Major Functions

1. Filter function
2. Immunologic function
3. Hematopoiesis function
4. Reservoir function

### Structures

1. **Red pulp:** 75% of the spleen. The sinuses account for 30% of the red pulp, they are lined by endothelial cells (littoral cells), which stain positive for factor VIII, CD8, sometimes CD4.

Red pulp functions: Filtration, form hematopoietic elements.

2. **White pulp:** 25% of the spleen. White pulp consists of B- and T-cells. B-cell area has germinal center, mantle zone and marginal zone. T-cell area is around the arterioles.

Germinal center: CD10+, CD19+, CD20+, CD79a+, high MIB-1, but CD5-, BCL2-.

*Mantle zone:* CD5+, IgM+, IgD+.

*White pulp functions:* Response to polysaccharide antigens stimulation.

### Indication for Splenectomy

1. Trauma/incidental
2. Hypersplenism
3. Hereditary spherocytosis
4. Autoimmune disorders
5. Splenic lipidoses
6. Myeloproliferative disorders
7. Lymphoproliferative disorders
8. Staging
9. Diagnosis of splenic mass.

### Causes of Splenic Reactive Follicular Hyperplasia

1. Children (normal).
2. Infections (Measles, Typhoid).
3. ITP, TTP.

4. Felty syndrome (rheumatoid arthritis, enlarged spleen, and low white blood count).
5. Acquired hemolytic anemia.
6. Acquired immunodeficiency syndrome.
7. Systemic Castleman's disease.

## *Splenic Lymphomas*

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### **Hematopoietic Neoplasms that may Involve Spleen**

#### ***Myeloproliferative Disorders***

1. Chronic myelogenous leukemia
2. Mastocytosis
3. Myelodysplasia
4. Myelofibrosis.

#### ***Lymphoproliferative Disorders***

1. Diffuse large B-cell lymphoma
2. Marginal zone B-cell lymphoma
3. Follicular lymphoma
4. Small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL)
5. Prolymphocytic leukemia
6. Mantle cell lymphoma
7. Hairy cell leukemia
8. Lymphoplasmacytic lymphoma
9. Plasmacytoma
10. Hodgkin's lymphoma
11. Hepatosplenic alpha-beta T-cell lymphoma
12. Hepatosplenic gamma-delta T-cell lymphoma
13. Angioimmunoblastic T-cell lymphoma
14. Peripheral T-cell lymphoma.

#### ***Others***

1. Histiocytic lymphoma/sarcoma
2. Follicular dendritic cell sarcoma
3. Interdigitating dendritic cell sarcoma
4. Langerhans cell histiocytosis
5. Castleman's disease

## Splenic B-cell Marginal Zone Lymphoma

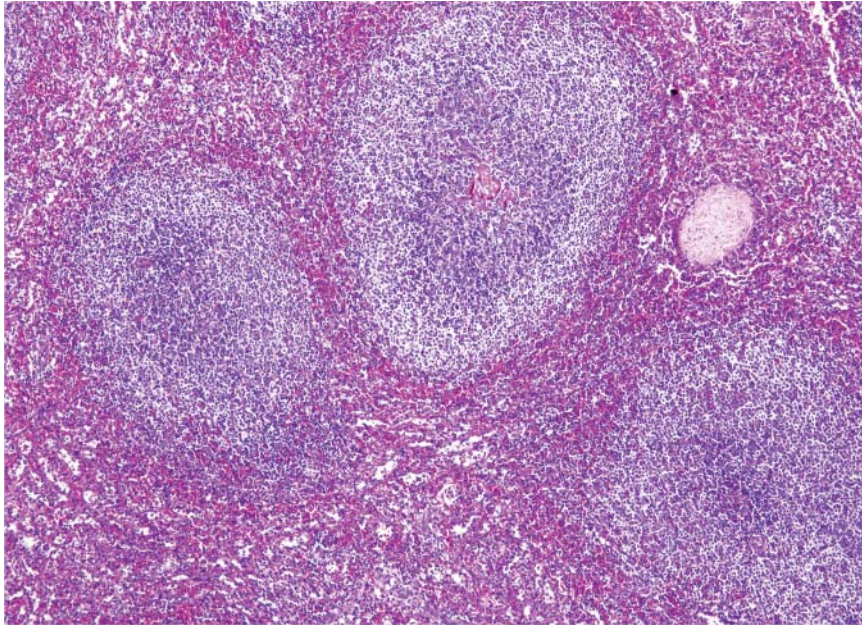
Splenic B-cell marginal zone lymphoma (SMZL) is a rare disorder, characterized by small lymphoma cells that surround and replace splenic white pulp and efface the mantle zone. Patients are usually > 50 years old, males and females are equally affected. Splenomegaly is common, however, lymphadenopathy is rare. Splenic B-cell marginal zone lymphoma may associate with autoimmune thrombocytopenia and/or anemia, or hepatitis C.

1. Peripheral blood: Anemia, thrombocytopenia. Circulating lymphoma cells are characterized by polar cytoplasmic projections (villi). As opposite to hairy cell leukemia these cytoplasmic villi are not circumferentially located around the cell.
2. Bone marrow is usually hypercellular with a variable increase in number of lymphocytes.
3. Spleen: Lymphoma involves both red and white pulp. The white pulp shows small lymphocytes replacing reactive germinal centers and effacement of the mantle zone (Figs 6-1A and B).
4. Flow cytometry: Lymphoma cells show expression of IgM, IgD, CD19, CD20, and clonal light chain. Unlike CLL/SLL, Splenic B-cell marginal zone lymphoma surface immunoglobulin IgD expression is bright and FMC7 is positive. Cyclin D1, CD5, CD10, CD11c, CD23, CD25, CD43, and CD103 are negative.
5. Cytogenetic and molecular study: Deletion of 7q31-32 (up to 40% of the cases, involving CDK6 gene), trisomy 3q and other abnormalities. However, translocation t(11;18)(q21;q21) of MALT type marginal zone lymphoma is extremely rare in SMZL.  
IgH gene rearrangement is positive.
6. Differential diagnosis: Immunophenotype comparisons of splenic marginal zone lymphoma and other B-cell lymphomas that may involve the spleen are listed in Table 6-1.

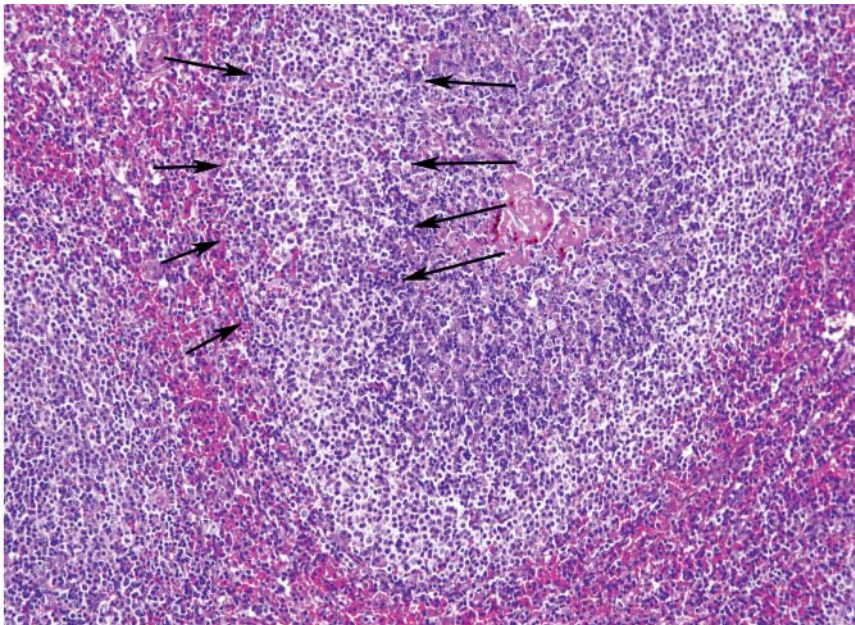
**TABLE  
6-1**

**Immunophenotype comparison of spleen B-cell lymphomas**

	CD5	CD10	CD23	CD11c	CD25	CD103
SMZL	-/+	-	-	+/-	-	-
CLL/SLL	+	-	+	-	-/+	-
Follicular lymphoma	-	+	-	-	-	-
Mantle cell lymphoma	+	-	-	-	-	-
Hairy cell leukemia	-	-	-	+	+	+



**Fig. 6-1A: Splenic marginal zone B-cell lymphoma.** White pulp showing expanded pale marginal zones at the periphery, with spread into the surrounding red pulp (Spleen section).



**Fig. 6-1B: Splenic marginal zone B-cell lymphoma.** At higher magnification, these cells have increased pale cytoplasm (so-called “monocytoid” B-cells) (Spleen section).

**Polyclonal B-cell lymphocytosis** is an uncommon disorder that occurs in middle-aged female smoker with persistent polyclonal lymphocytosis (binucleated lymphocytes in peripheral blood and lymphocytes in the interstitial of bone marrow, and mimics SMZL in the spleen). It has a benign clinical course.

### Splenic Diffuse Red Pulp Small B-cell Lymphoma (WHO 2008 Splenic B-cell Lymphoma/Leukemia, Unclassifiable, Provisional Entity)

Splenic diffuse red pulp small B-cell lymphoma is an uncommon lymphoma characterized by small monomorphous B lymphocytes diffusely involving red pulp. Involvement of lymph nodes and lymphocytosis in the peripheral blood is rare.

1. Peripheral blood and bone marrow: Lymphoma cells are present in the peripheral blood with villous cytology and involve the sinus of bone marrow.
2. Flow cytometry: Diagnosis should exclude CLL, hairy cell leukemia, lymphoplasmacytic lymphoma, and prolymphocytic leukemia (Table 6-2).

**TABLE  
6-2**

Immunophenotype comparison of splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia and marginal zone lymphoma

	CD11c	CD25	CD103	DBA.44	TRAP
Hairy cell leukemia	+	+	+	+	+
Hairy cell leukemia variant	+	–	+	+	–
SMZL	+/-	–	-/+	-/+	–
Splenic diffuse red pulp small B-cell lymphoma	–	–	–	–	–

### Hepatosplenic T-cell Lymphoma

Hepatosplenic T-cell lymphoma is a rare  $\gamma/\delta$  peripheral T-cell lymphoma involving sinusoidal areas of the liver, red pulp of spleen and bone marrow (less common). Peak incidence occurs in adolescents and young adults (median age around 35 years old), with a male predominance. The patients usually present as hepatosplenomegaly without lymphadenopathy. Up to 20% of hepatosplenic T-cell lymphomas arise in patients who are on immunosuppressive therapy.

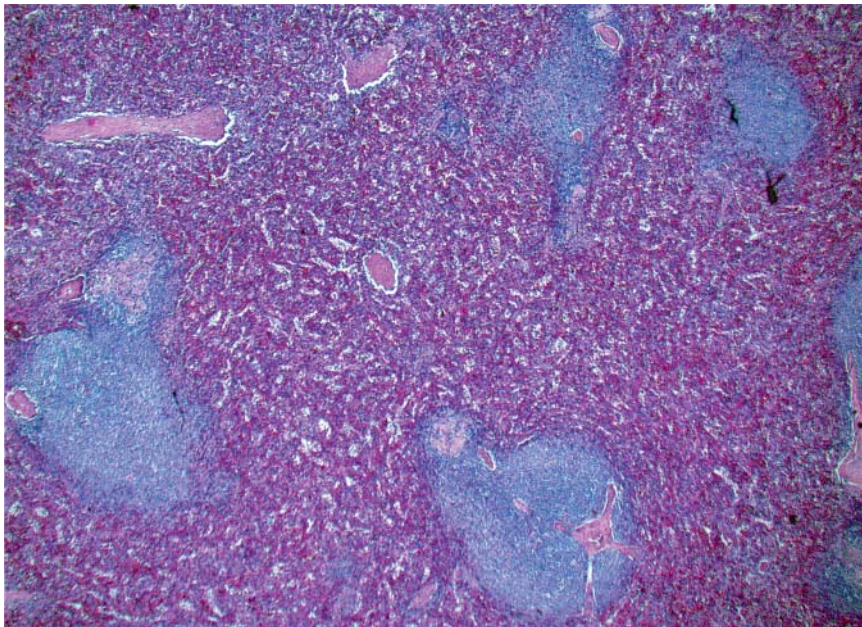


1. Peripheral blood: Anemia and leukopenia. Involvement is uncommon at presentation.
2. Bone marrow: Constantly involved, but difficult to identify without the aid of immunohistochemical stains or flow cytometry study.
3. Flow cytometry and immunophenotype: Resembles the normal  $\gamma/\delta$  T-cell (CD2+, CD3+, CD7+, CD11c+,  $\gamma/\delta$ +, TIA+, CD8-/+, CD4-,  $\beta$ F1-, and perforin-), EBV-. Rare cases are ( $\alpha/\beta$ )+, ( $\gamma/\delta$ )-.
4. Cytogenetic and molecular studies: **Isochromosome 7q** is present in most cases.

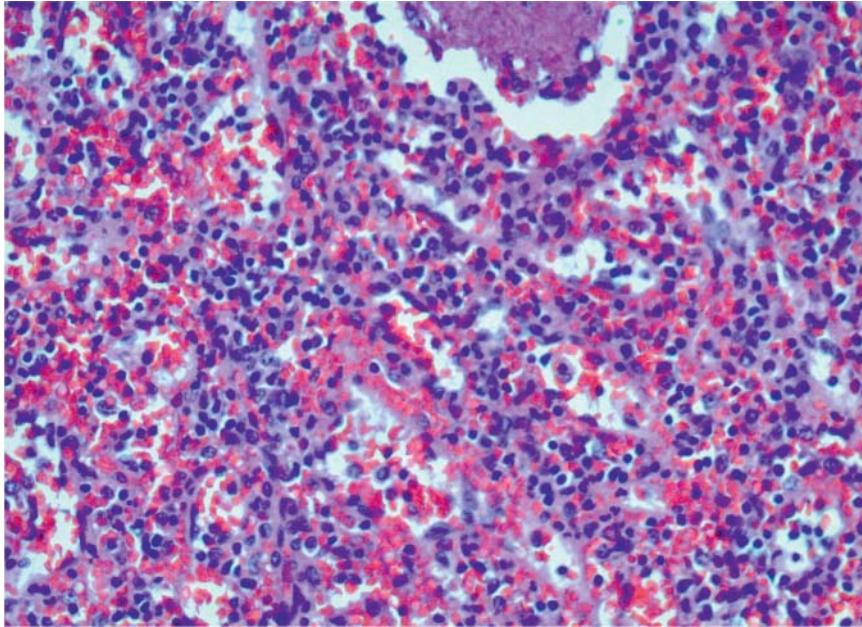
TCR gene rearrangement is positive (Figs 6-2A to E).

### Other Lymphomas that may Involve Spleen

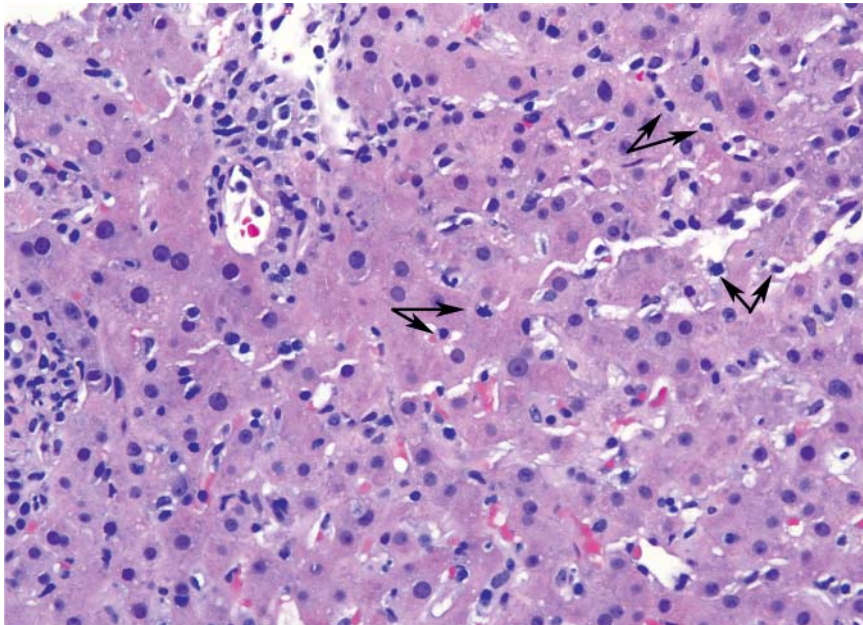
Please see chapters 9 and 10.



**Fig. 6-2A: Hepatosplenic T-cell lymphoma.** Spleen section showing expansion of red pulp relatively preserved white pulps (Spleen section).

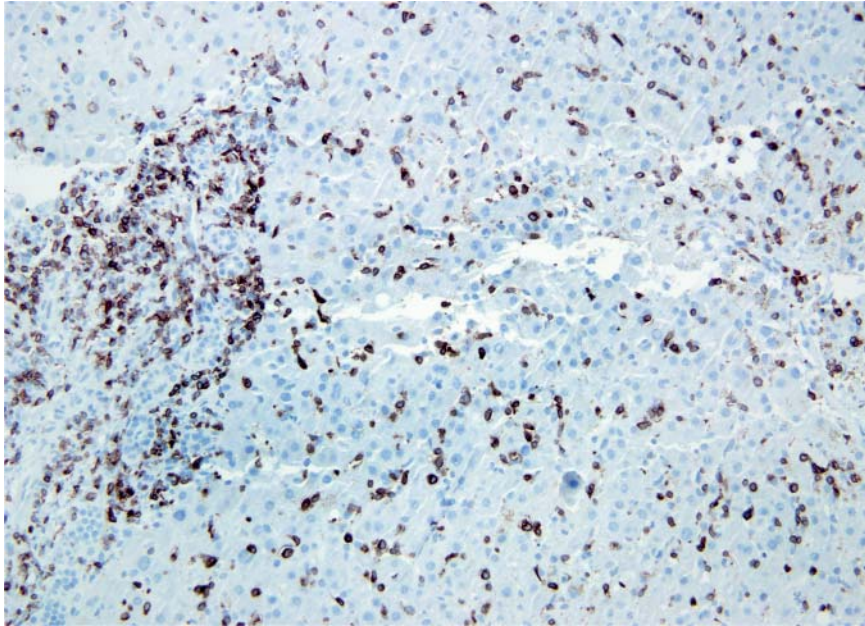


**Fig. 6-2B: Hepatosplenic T-cell lymphoma.** Spleen red pulp section showing monotonous population of lymphocytes with medium-sized nuclei and a rim of pale cytoplasm (Spleen section).

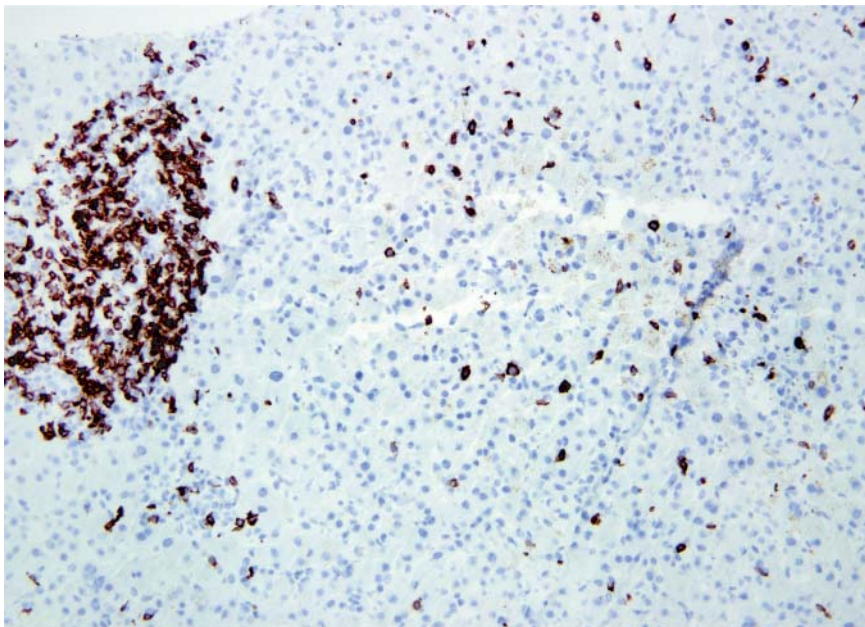


**Fig. 6-2C: Hepatosplenic T-cell lymphoma.** Liver section showing small lymphocytes infiltrating the sinusoids (arrow) (Liver section).





**Fig. 6-2D: Hepatosplenic T-cell lymphoma.** Immunohistochemical stain for CD3 showing neoplastic T-cells infiltrating the sinusoids of the liver (Liver section).



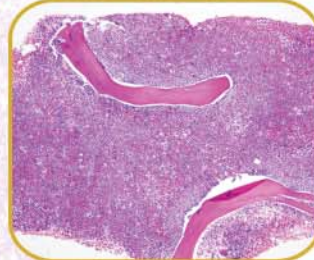
**Fig. 6-2E: Hepatosplenic T-cell lymphoma.** Immunohistochemical stain for CD5 showing that the neoplastic T-cells dropped this pan T-cell marker (compare to Fig. 6-2D in the same area) (Liver section).



CHAPTER

7

# Myelodysplastic Syndromes



## *Myelodysplastic Syndromes (MDS)*

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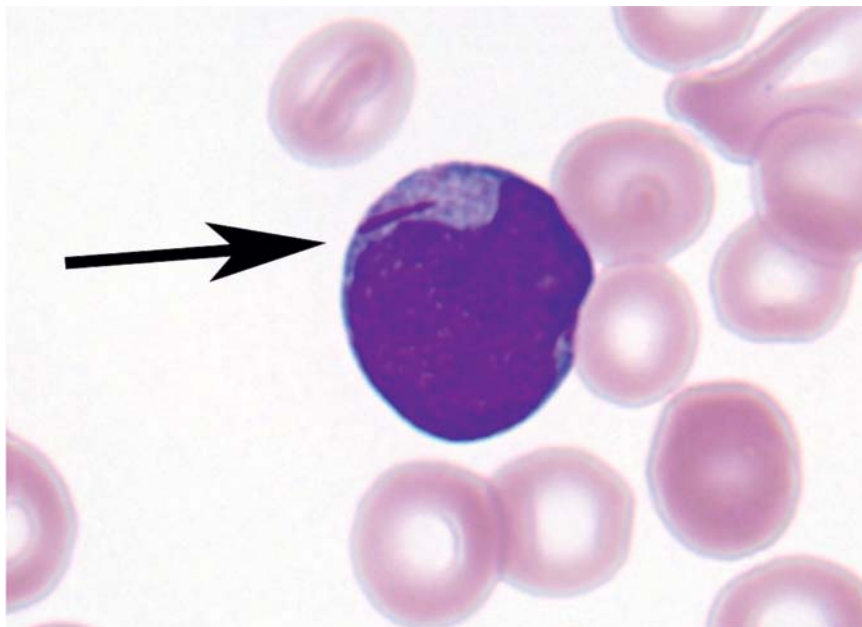
### **WHO Classification of MDS**

1. Refractory cytopenias with unilineage dysplasia (RCUD):
    - a. Refractory anemia (RA).
    - b. Refractory neutropenia (RN).
    - c. Refractory of thrombocytopenia (RT).
  2. Refractory anemia with ringed sideroblasts (RARS).
  3. Refractory cytopenia with multilineage dysplasia (RCMD).
  4. Refractory anemia with excess blasts (RAEB)
    - a. RAEB1: 5-9% blasts in blood or marrow.
    - b. RAEB2: 10-19% blasts in blood or marrow.
  5. Myelodysplastic syndrome associated with isolated del(5q).
  6. Myelodysplastic syndrome-unclassified (MDS-U).
- Note: (all the above have less than 1000/ $\mu$ l monocytes in the blood)

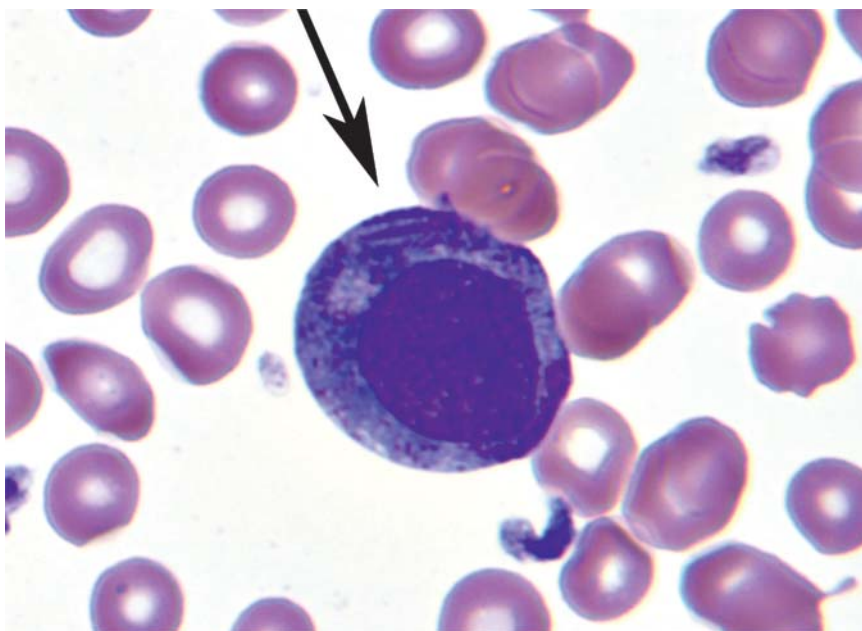
1. **RCUD:** Predominantly RA, usually advanced age, RN and RT are rare
  - a. Anemia, reticulocytopenia.
  - b. Bone marrow erythroid hyperplasia.
  - c. Less than 15% ringed sideroblasts.
  - d. Less than 5% blasts in marrow, none in the peripheral blood.
  - e. Less than 1000/ $\mu$ l monocytes in the peripheral blood.
2. **RARS:** Similar to RA except  $\geq 15\%$  ringed sideroblasts
  - a. Usually erythroid dysplasia only.
  - b. Dimorphic anemia is common.

(WHO definition of ringed sideroblasts: erythroid precursors with one-third or more of the nucleus encircled by 10 or more siderotic granules on an Iron stained aspirate smear).
3. **RCMD:** Bi- or pancytopenia
  - a. Dysplasia seen in  $\geq 10\%$  cells in two or more lineages.
  - b. 15% or more ringed sideroblasts may be present.
  - c. Less than 5% blasts in the bone marrow, none or rare in the peripheral blood.
  - d. Less than 1000/ $\mu$ l ( $< 1 \times 10^9$ /L) monocytes in the peripheral blood no Auer rod.
4. **RAEB:** Less than 1000/ $\mu$ l ( $< 1 \times 10^9$ /L) monocytes in the peripheral blood
  - a. **RAEB1:** 5-9% blasts in the peripheral blood or bone marrow (if Auer rod present, call it RAEB2, Figs 7-1A and B).
  2. **RAEB2:** 10-19% blasts in the peripheral blood or bone marrow





**Fig. 7-1A: Auer rod** in a blast cell. Auer rod is a long slender inclusion in the cytoplasm of leukemia cells (Peripheral blood smear).



**Fig. 7-1B: Auer rod** in a blast cell of a patient with APL. Cell contains multiple Auer rods have been referred to as a “faggot cell”. Faggot means a bundle of sticks (Peripheral blood smear).

**5. Myelodysplastic syndrome with isolated del(5q)**

del(5q) is the sole cytogenetic abnormality and characterized by anemia with/without other cytopenia and/or thrombocytosis. It has a stable clinical course. Blast count is <5% in the bone marrow and 1% in the peripheral blood. Auer rods are absent.

Bone marrow biopsy is usually hypercellular or normocellular with frequently erythroid hyperplasia and increased megakaryocytes. Megakaryocytes are normal to slightly decreased in size with conspicuously non-lobated and hypolobated nuclei. Erythroid and myeloid lineage dysplasia are uncommon.

The prognosis of MDS is related to patient's age, peripheral blood and bone marrow and cytogenetic findings (Table 7-1).

**TABLE 7-1**  
**Prognosis in MDS**

Good	Poor
Young age Normal or reduced neutrophil and platelets No Auer rods Ringed sideroblasts present Normal karyotype Isolated deletion of 5q and 20q Deletion of Y	Advanced age Neutropenia or thrombocytopenia Auer rods present Absent ringed sideroblasts Complex karyotype, chromosome 7 abnormality

## ***Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN)***

### **WHO Classification of Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN)**

1. Chronic myelomonocytic leukemia (CMML)
2. Atypical chronic myeloid leukemia (aCML), BCR-ABL negative
3. Juvenile myelomonocytic leukemia (JMML)
4. Myelodysplastic/Myeloproliferative neoplasm, unclassifiable (MDS/MPN, U)

### **CMML**

Morphologic features are similar to RA, RCMD or RAEB but have more than 1000/ $\mu$ L ( $>1 \times 10^9$ /L) monocytes in peripheral blood. Organomegaly is

more common than in MDS. Leukemic cells involve the red pulp of spleen. Prognosis: the percentage of blasts in the peripheral blood and in the bone marrow is the most important factor in determining survival.

### ***WHO Diagnostic Criteria for CMML***

1. Persistent peripheral blood monocytosis  $>1000/\mu\text{l}$  ( $>1 \times 10^9/\text{L}$ ).
2. No Philadelphia chromosome or BCR-ABL fusion gene.
3. No rearrangement of PDGFRA or PDGFRB.
4. Less than 20% blasts (include myeloblasts, monoblasts and promonocytes) in the blood and in the bone marrow.
5. Dysplasia in one or more myeloid lineages, if dysplasia is absent or minimal, the following criteria must meet:
  - Cytogenetic or molecular genetic abnormalities
  - Monocytosis persisting for at least 3 months
  - All other causes of monocytosis have been excluded.

### ***Diagnostic Criteria for CMML 1 and CMML 2***

1. **CMML 1:** Blasts (including promonocytes)  $<5\%$  in peripheral blood, and  $<10\%$  in bone marrow.
2. **CMML 2:** Blasts (including promonocytes) 5-19% in peripheral blood, and 10-19% in bone marrow or Auer rods present irrespective of blast count.
3. CMML expressing p190 BCR-ABL1 should be classified as CML. If the cytogenetic study for t(9;22)(q34;q11) is negative, both p210 and p190 should be checked by PCR.
4. CMML with i(17q): dysgranulopoiesis with severe hyposegmentation of neutrophil nuclei and a high risk of transformation to AML (some case may categorized as MDS/MPN).
5. Cytogenetics abnormalities present in 20-40% of CMML cases, including +8, -7, 7q-, abnormalities of 12p, and occasionally t(5;12)(q33;p13). Approximately 40% cases have RAS mutations, and 13% cases have JAK2 V617F mutations.

### **Atypical Chronic Myeloid Leukemia**

Atypical chronic myeloid leukemia (aCML) has features similar to CML but basophilia are minimal or lacking. Neutrophil dysplasia and thrombocytopenia are prominent. The t(9;22) Philadelphia chromosome and BCR-ABL fusion gene are **absent**.

**1. WHO Diagnostic Criteria for aCML**

- a. Persistent peripheral blood leukocytosis ( $\text{WBC} \geq 13 \times 10^9/\text{L}$ ).
  - b. No t(9;22) Philadelphia chromosome or BCR-ABL fusion gene.
  - c. No rearrangement of PDGFRA or PDGFRB.
  - d. Neutrophil precursors (promyelocytes, myelocytes, metamyelocytes)  $\geq 10\%$  of leukocytes.
  - e. Minimal absolute basophilia, basophils usually  $< 2\%$  of leukocytes.
  - f. No or minimal absolute monocytosis, monocytes usually  $< 10\%$  of leukocytes.
  - g. Hypercellular bone marrow with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages.
  - h. Less than 20% blasts in the peripheral blood and in the bone marrow.
- 2.** The most common cytogenetic abnormalities are +8 and del(20q).
- 3.** Prognosis
- a. Adverse prognostic factors include:
    - Age  $> 65$
    - Female
    - $\text{WBC} > 50 \times 10^9/\text{L}$
    - Thrombocytopenia
    - $\text{Hb} < 10 \text{ g/dL}$
  - b. Bone marrow transplant may improve outcome. 15-40% of the patients will evolve to AML, the rest succumb to bone marrow failure.

**MDS in Children**

MDS in children is uncommon, comprising approximately 3-9% of pediatric hematologic malignancies.

Pediatric MDS includes JMML and the transient myeloproliferative disorder (associated with Down's syndrome).

**1. Diseases associated with MDS:**

- a. Fanconi anemia
  - b. Down syndrome
  - c. Neurofibromatosis type 1 (NF1)
  - d. Kostmann syndrome
  - e. Shwachman-Diamond syndrome
  - f. Blackfan-Diamond anemia
  - g. Bloom syndrome.
- 2.** Adult-type MDS in children:
- a. Monosomy 7 or del 7q common
  - b. Monosomy 5 or del 5q uncommon (common in adult).

**Juvenile Myelomonocytic Leukemia (JMML)**

JMML previously described as juvenile chronic myeloid leukemia (JCML), chronic myelomonocytic leukemia (CMML) and infantile monosomy 7-syndrome.

1. 75% cases of JMML are less than 3 years old.
2. 10-15% occurs in patients with neurofibromatosis type 1 (NF1).
3. Male to female ratio is approximately 2 to 1.
4. Clinical features include hepatosplenomegaly, lymphadenopathy, recurrent infections, and bleeding.
5. Cytogenetic and molecular studies:
  - 25% of the cases have isolated monosomy 7.
  - 10% have other abnormalities.
  - 65% have normal karyotype.

**WHO Diagnostic Criteria for JMML**

1. Peripheral blood monocytosis  $>1000/\mu\text{l}$  ( $1 \times 10^9/\text{L}$ ).
2. Blasts and promonocytes  $< 20\%$  of the leukocytes in the peripheral blood and in the bone marrow.
3. No Philadelphia chromosome or BCR/ABL fusion gene.
4. Plus two or more of the following:
  - Increased hemoglobin F for age
  - Immature granulocytes in the blood
  - Leukocyte count  $>10 \times 10^9/\text{L}$
  - Clonal chromosome abnormality (may be monosomy 7)
  - GM-CSF hypersensitivity of myeloid progenitors *in vitro*.

**Prognosis of JMML**

1. Good:
  - Younger than 1 year old.
2. Poor:
  - Low platelets ( $< 33\text{K}/\mu\text{l}$ )
  - Elevated hemoglobin F ( $>15\%$ )
  - Two or more cytogenetic clonal abnormalities.

**Myelodysplastic Syndrome/Myeloproliferative Neoplasm, Unclassifiable (MDS/MPN, U)**

The cases exhibit features of both MDS and MPN, but not fulfill the criteria for any of the other disorders in the MDS/MPN group.



## *Myeloproliferative Neoplasms*

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### **WHO Classification of Myeloproliferative Neoplasms (MPN)**

1. Chronic myelogenous leukemia (BCR/ABL positive CML).
2. Chronic neutrophilic leukemia (CNL).
3. Polycythemia vera (PV).
4. Primary myelofibrosis (PMF).
5. Essential thrombocythemia (ET).
6. Chronic eosinophilic leukemia, NOS (CEL, NOS).
7. Mastocytosis.
8. Myeloproliferative neoplasm, unclassifiable (MPN, U).

### **Chronic Myelogenous Leukemia (CML)**

The hallmark of CML is the **Philadelphia chromosome (Ph)**. Translocation of t(9;22)(q24;q11.2) chromosome results in **BCR/ABL** fusion gene. This gene fusion is diagnostic for CML. The BCR/ABL gene fusion leads to the formation of oncoprotein P210 (99% cases of the CML), P190 (rare in CML, and common in ALL) and P230 (CML, rare).

CML is divided into three phases: chronic, accelerated and blast phase.

#### **Chronic Phase of CML**

1. Clinical features: Fatigue, weight loss, fever, and splenomegaly. 20-40% of cases are asymptomatic.
2. Peripheral blood: Leukocytosis, basophilia with or without thrombocytosis. Granulocyte maturation is “bulge” at myelocyte, band and segmented stages. Blasts are less than 2%. No significant dysplasia is present.
3. Bone marrow: Hypercellular (> 90% cellularity). The thickened layers of immature granulocytes are about 5-6 cells deep (normal 2-3 cells). Megakaryocytes are small (“**dwarf**” megakaryocytes) with hypolobulated nuclei and arranged in clusters. Pseudo-Gaucher cells are commonly observed; they are histiocytes derived from Ph<sup>+</sup> clones secondary to the excessive phospholipid released from granulocytes. M:E ratio is increased. Blasts are usually less than 5%, but always less than 10%. Reticulin fibrosis is variable, from none to moderate.
4. Cytogenetic and molecular studies: Philadelphia chromosome, t(9;22)(q24;q11.2) - BCR/ABL fusion gene. **BCR-ABL gene fusion t(9;22)(q34;q11.2) is a diagnostic requirement for CML.**

The breakpoint cluster region (BCR) gene is located on chromosome 22. The Abelson (ABL) proto-oncogene is located on chromosome 9.

This translocation results in three different lengths of fusion protein products. The BCR-ABL gene fusion results in unregulated tyrosine kinase activity, an increased cell proliferation and a decreased apoptosis. There are three BCR-ABL gene fusion protein products:

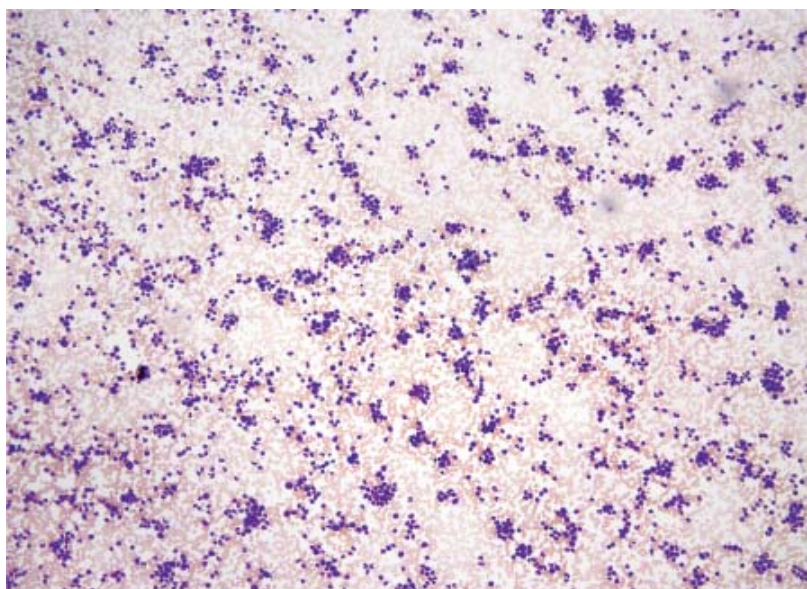
- P190: 50-80% ALL (adult), 90% ALL (children), rare in CML (may have CMML monocytosis feature)
- P210: > 90% CML, 20-30% ALL (adult), 10% ALL (children)
- P230: rare, associated with chronic neutrophilic leukemia.

Chromosome karyotyping, FISH and qualitative RT-PCR have been used for detecting BCR-ABL fusion gene (Figs 7-2A to D).

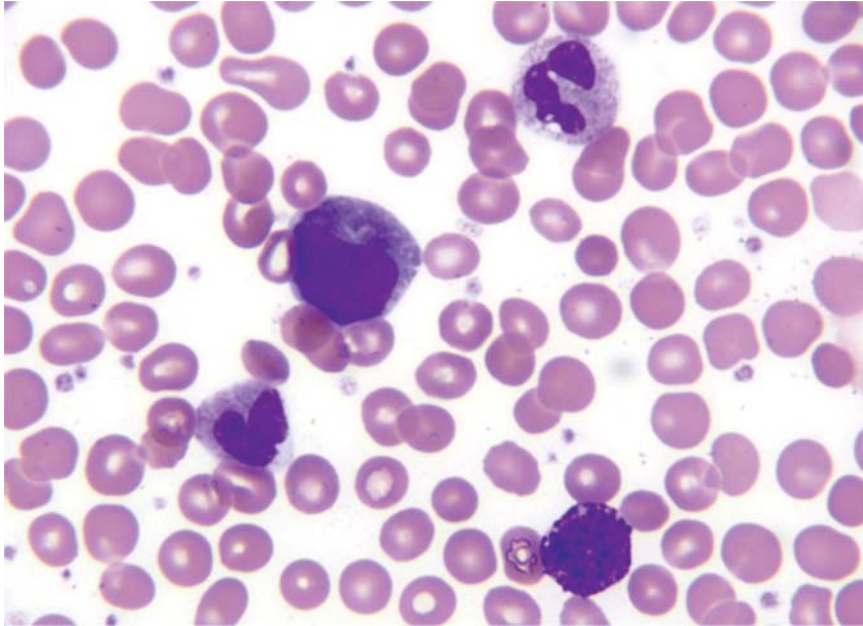
### ***Accelerated Phase CML Diagnostic Criteria***

Diagnose as accelerated phase of CML if any of the following are present:

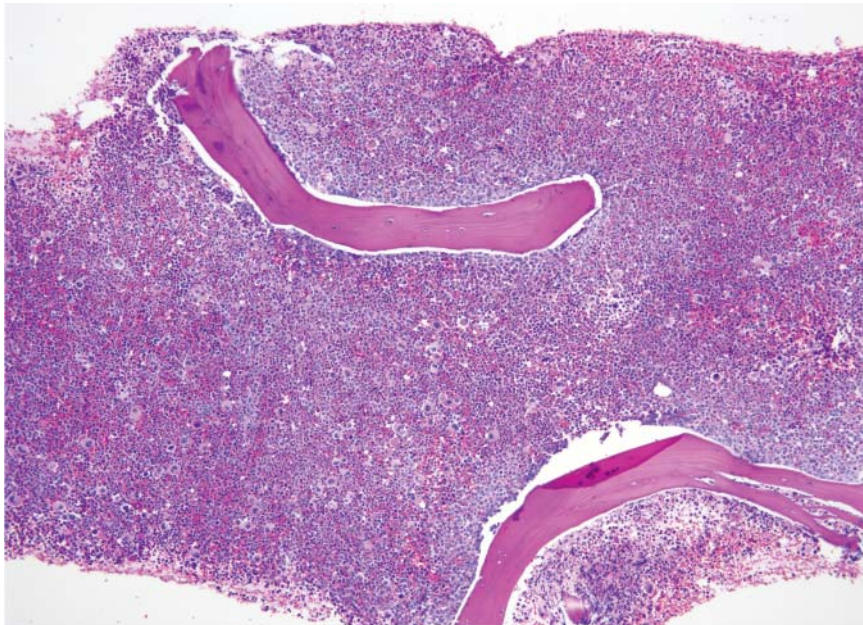
1. Blast count is 10-19% in peripheral blood or in bone marrow.
2. Persistent thrombocytopenia  $<100 \times 10^9/L$  unrelated to therapy.
3. Persistent thrombocytosis  $>1000 \times 10^9/L$  uncontrolled by therapy.
4. Persistent WBC count  $>10 \times 10^9/L$  or increasing spleen size and unresponsive to therapy.
5. Cytogenetic study shows evidence of clonal evolution occurring after initial diagnostic karyotype.
6. 20% or more basophils in the peripheral blood.



**Fig. 7-2A: Chronic myelogenous leukemia.** Marked leukocytosis (Peripheral blood smear).

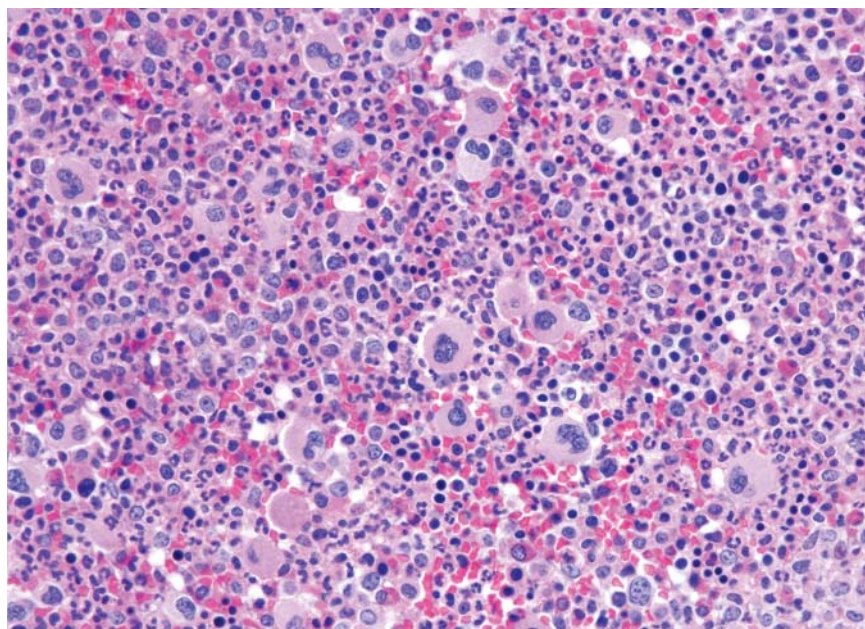


**Fig. 7-2B: Chronic myelogenous leukemia.** Basophilia is common in CML (Peripheral blood smear).



**Fig. 7-2C: Chronic myelogenous leukemia.** Markedly hypercellular bone marrow near 100% (Bone marrow section).





**Fig. 7-2D: Chronic myelogenous leukemia.** At higher magnification, showing megakaryocytes and myeloid cells are the dominant cells. Megakaryocytes tend to be small (Bone marrow section).

### ***Blast Phase (Acute Leukemia Phase) Diagnostic Criteria***

1. Blasts  $\geq 20\%$  of the peripheral WBC or nucleated cells of the bone marrow.
2. Extramedullary blast proliferation (myeloid or lymphoid).

In most cases of blast phase CML, the blasts are myeloid lineage, however, about 20-30% of the cases, the blasts will be lymphoblasts with a precursor B-cell phenotype. The morphologic clue to identify CML blast phase is **basophilia in a background of AML or ALL**.

### ***Leukocyte Alkaline Phosphatase (LAP) Score and MPN***

In case of reactive leukocytosis (with the normal intact enzyme) the LAP score is normal or above normal. In contrast, the neutrophils of chronic phase CML are deficient in the enzyme, so the LAP score is low. In CML the LAP score is below normal (near zero) which is not specific for CML, as there are other diseases that may have low or high LAP scores. When CML is in remission, the LAP score may be normal. Cytogenetic and molecular studies and FISH have replaced the use of LAP in the diagnosis of CML.

**1. Low LAP score:**

- Chronic phase CML
- Paroxysmal nocturnal hemoglobinuria
- Pernicious anemia
- Hypophosphatemia
- Some MDS.

**2. Normal or high LAP score:**

- Reactive leukocytosis
- Accelerated or blast phase CML
- Polycythemia vera.

***Differential Diagnosis of Chronic Phase CML***

Reactive leukocytosis/Leukemoid reaction, aCML and CMML.

**Chronic Neutrophilic Leukemia**

Chronic neutrophilic leukemia (CNL) shows sustained peripheral blood neutrophilia. The neutrophils are primarily mature segmented neutrophils, toxic granules and Döhle bodies may present.

- There is no basophilia, monocytosis or eosinophilia.
- Bone marrow is hypercellular due to neutrophilic proliferation.
- Hepatosplenomegaly is common.
- Philadelphia chromosome or BCR/ABL fusion gene is absent. Del(11q), del(12p) and trisomy 21 may be present.

***WHO Diagnostic Criteria for Chronic Neutrophilic Leukemia***

1. Peripheral blood leukocytosis  $WBC \geq 25 \times 10^9/L$  (segmented/band  $>80\%$ , pro/myelo/metamyelocytes  $< 10\%$ , blast  $< 1\%$ ).
2. Hypercellular bone marrow (blast  $< 5\%$ , increase neutrophilic granulocytes with normal maturation, megakaryocytes are normal or left shifted).
3. Hepatosplenomegaly.
4. No identifiable cause for physiologic neutrophilia (no infectious or inflammatory process, no underlying tumor).
5. No Philadelphia chromosome or BCR-ABL fusion gene.
6. No rearrangement of PDGFRA, PDGFRB or FGFR1.
7. No evidence of PV, PMF or ET.
8. No evidence of MDS or MDS/MPN (no granulocytic dysplasia, no myelodysplastic changes in other myeloid lineage, monocytes  $< 1 \times 10^9/L$ ).



## Polycythemia Vera

Polycythemia vera (PV) is an acquired clonal primary polycythemic disorder. The peak age is 60s, male to female ratio is 2:1. Splenomegaly is a common clinical presentation.

Polycythemia vera is due to mutations in a multipotential hematopoietic stem cell, which results in an excess production of functionally normal erythrocytes, a variable overproduction of granulocytes and monocytes, and of platelets. **Low serum erythropoietin (EPO) is hallmark of Polycythemia Vera.** EPO is a glycoprotein produced mainly in the kidney. In polycythemia vera, the EPO level is extremely low. In secondary polycythemia, the EPO level is elevated (Table 7-2).

The majority of patients (> 95%) have somatic mutations of the JAK2 V617F. JAK2 V617F mutation is not specific for polycythemia vera, this mutation is also observed in essential thrombocytosis, primary myelofibrosis and other hematologic neoplastic disorders but is less common than in polycythemia vera.

Polycythemia vera can undergo a clonal evolution to primary myelofibrosis and acute leukemia.

### WHO Diagnostic Criteria for Polycythemia Vera

The diagnosis requires two major criteria plus one minor criteria **or** first major plus two minor criteria.

#### 1. Major

- a. Hb > 18.5 g/dl in men, >16.5 g/dl in women or elevated RBC mass >25% above the mean normal predicted value.
- b. Presence of JAK2 V617F or other functionally similar JAK2 mutation.

#### 2. Minor

- a. Bone marrow biopsy showed panmyelosis with prominent erythroid and megakaryocytes proliferation.
- b. Serum erythropoietin level below the normal reference range.
- c. Endogenous erythroid colony formation *in vitro*.

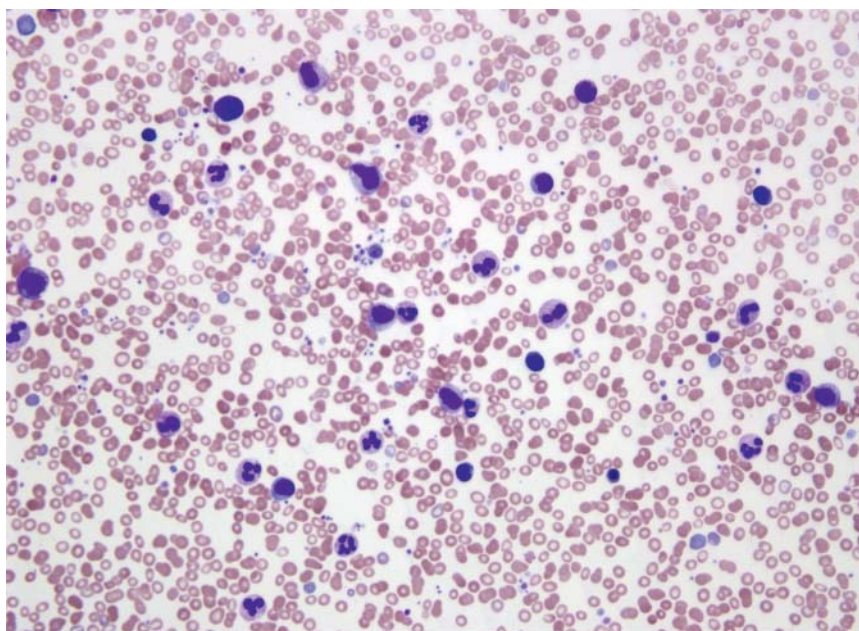
### WHO Diagnostic Criteria for Post-polycythemic Myelofibrosis

#### 1. Required criteria

- a. Documentation of a previous diagnosis of WHO-defined polycythemia vera.
- b. Bone marrow fibrosis grade 2-3 (on a 0-3 scale) or grade 3-4 (on a 0-4 scale).

**TABLE  
7-2****Comparison of polycythemia vera and secondary erythrocytosis**

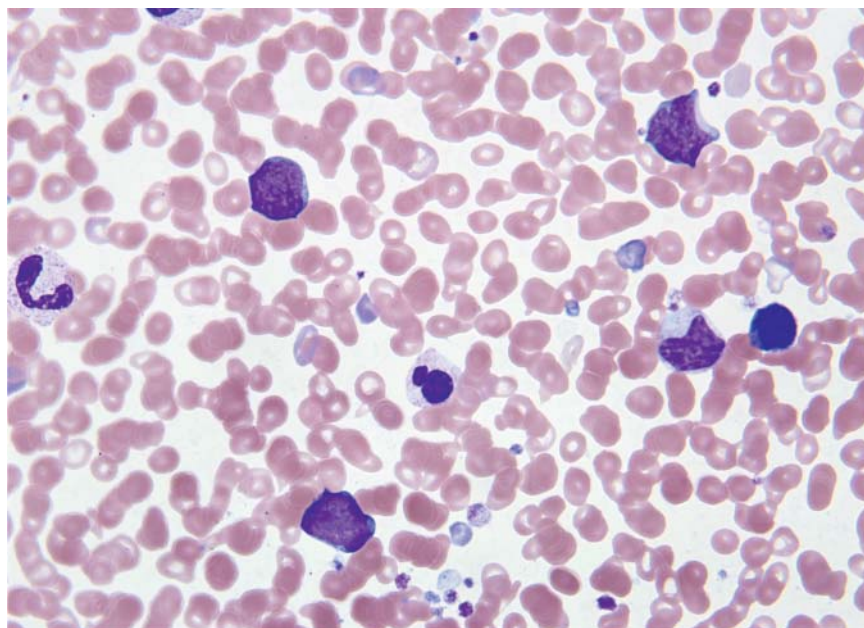
Findings	PV	2nd erythrocytosis
RBC mass	increased	increased
Leukocytosis	present in >50%	usually absent
Thrombocytosis	present in >60%	usually absent
Organomegaly	present in >70%	absent
Serum erythropoietin	decreased	normal
PaO <sub>2</sub> /SaO <sub>2</sub>	normal	decrease in hypoxia-related
Uric acid	increased	normal
Marrow proliferation	trilineage	erythroid
Marrow iron storage	absent	normal
Mpl protein expression	reduced expression	normal
PRV-1 protein expression	overexpression	normal
JAK2 mutation	present in >90%	negative



**Fig. 7-3A: Polycythemia vera.** Post-polycythemic myelofibrosis. Red blood cell anisopoikilocytosis, occasional teardrop cells, neutrophilia, and nucleated red blood cells (Peripheral blood smear).

## 2. Additional criteria (two are required)

- a. Anemia, or sustained loss of either phlebotomy or cytoreductive treatment requirement for erythrocytosis.



**Fig. 7-3B: Polycythemia vera.** Post-polycythemic myelofibrosis. Blasts present in the peripheral blood (Peripheral blood smear).

- b. Leukoerythroblastic peripheral picture
- c. Increasing splenomegaly
- d. Development of more than one of the three constitutional symptoms:  
>10% weight loss in 6 months, night sweats, and unexplained fever.

## Primary Myelofibrosis

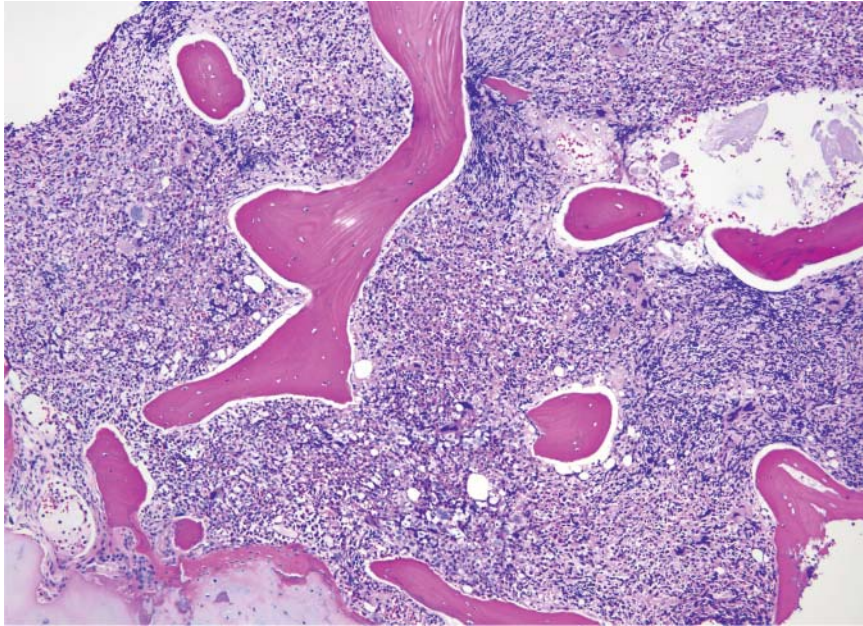
Primary myelofibrosis (PMF) is a rare clonal disease, characterized by marked bone marrow granulocytic and atypical megakaryocytic proliferation, ineffective erythropoiesis, gradual deposition of reticulin fibers, and progressing to fibrosis and acute leukemia.

The fibroblasts in primary myelofibrosis and other myeloid proliferative neoplasms are not derived from neoplastic clones. The myelofibrosis is reactive, secondary to the release of fibrogenic factor (PDGF, TGF $\beta$ ) from granulocytes and megakaryocytes.

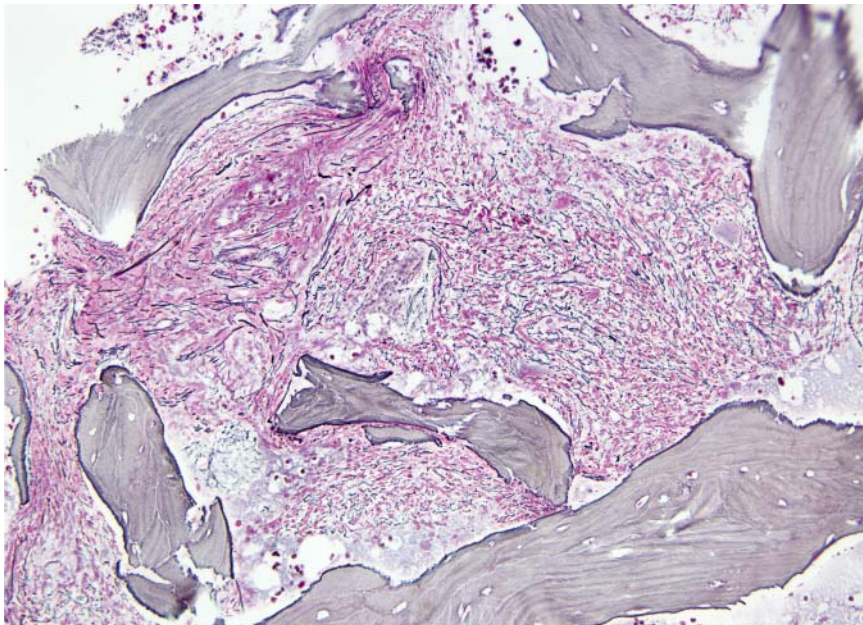
Cytogenetic abnormalities may be present, none of them is specific for primary myelofibrosis.

The level of Mpl, a thrombopoietin receptor on megakaryocytes and stem cells, is decreased. Decreased level of Mpl is also observed in polycythemia vera or essential thrombocythemia (Figs 7-4A and B).





**Fig. 7-4A: Primary myelofibrosis.** Increased collagen fibrosis with decreased hematopoietic elements and dysplastic megakaryocytes (Bone marrow section).



**Fig. 7-4B: Primary myelofibrosis.** Reticulin fiber stain shows increased and thickened fibrils representing collagen type III (Bone marrow section).

**WHO diagnostic criteria for primary myelofibrosis require meeting all three major and two minor criteria**

**1. Major criteria**

- a. Presence of megakaryocyte proliferation and atypia, usually accompanied by reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e. prefibrotic cellular phase disease).
- b. Not meeting WHO criteria for PV, BCR-ABL positive CML, MDS, or other myeloid neoplasms.
- c. Demonstration of JAK2 **V617F** mutation or other clonal marker (e.g. MPL W515K/L), or, in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases.

**2. Minor criteria**

- a. Leukoerythroblastosis seen in peripheral blood
- b. Increase in serum lactate dehydrogenase (LDH) level
- c. Anemia
- d. Splenomegaly.

## **Essential Thrombocythemia and Thrombocytosis**

Essential thrombocythemia (ET) is a myeloproliferative neoplasm distinguished from polycythemia vera and primary myelofibrosis by the absence of polycythemia and marrow fibrosis at presentation.

Essential thrombocythemia is often diagnosed following the incidental finding of a high platelet count, and small proportion of patients present with thrombotic or hemorrhage complications. Splenomegaly is usually mild, if significant splenic enlargement is present, the possibility of another myeloproliferative neoplasm such as primary myelofibrosis or chronic myeloid leukemia should be considered.

Thrombotic complications are the major source of morbidity and mortality in essential thrombocythemia.

### ***WHO Criteria for Essential Thrombocythemia (Diagnosis Requires Meeting all Four Criteria)***

1. Sustained platelet count  $> 450 \times 10^9/L$ .



2. Bone marrow biopsy showing proliferation of the megakaryocytic lineage with increased number of enlarged mature megakaryocytes. No significant increase of left-shift of neutrophil granulopoiesis or erythropoiesis.
3. Not meeting WHO criteria for PV, PMF, BCR-ABL positive CML, or MDS, or other myeloid neoplasms.
4. Demonstration of JAK2 V617F or other clonal marker, or in the absence of JAK2 V617F mutation, no evidence for reactive thrombocytosis (see below).

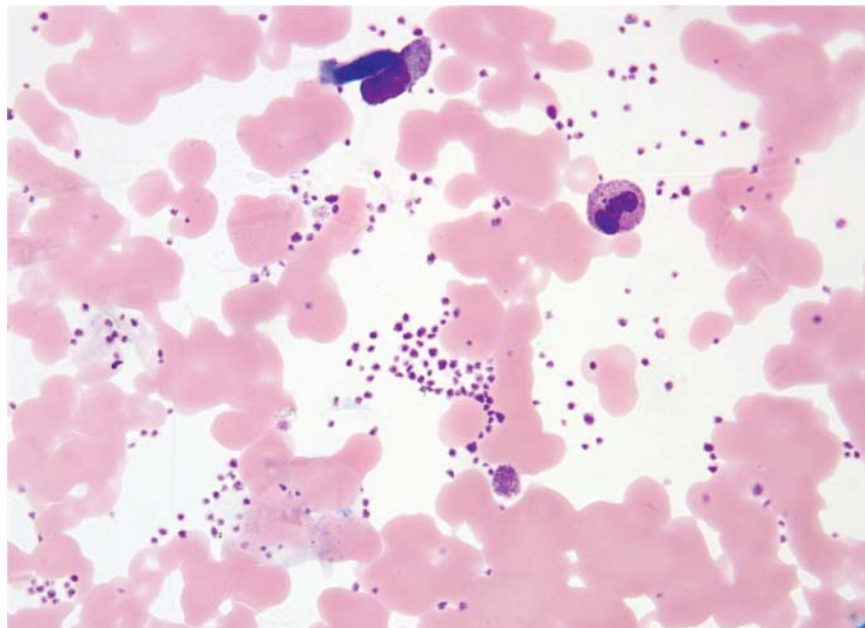
### ***Causes of Thrombocytosis***

1. Reactive thrombocytosis
  - Infection
  - Inflammatory disease (inflammatory bowel disease, collagen vascular disease)
  - Post splenectomy
  - Non-hematopoietic malignancy
  - Hemorrhage
  - Chronic iron deficiency anemia
  - Trauma (especially brain injury)
2. Hematopoietic neoplasms associated with thrombocytosis
  - Chronic myeloproliferative disease
  - Polycythemia vera
  - Primary myelofibrosis (prefibrotic stage)
  - Essential thrombocythemia
  - AML associated with t(3;3)(q21 q26) or inv(3)(q21q26)
  - MDS associated with del(5q)
  - Other MDS/MPD (sideroblastic anemia with marked thrombocythemia).

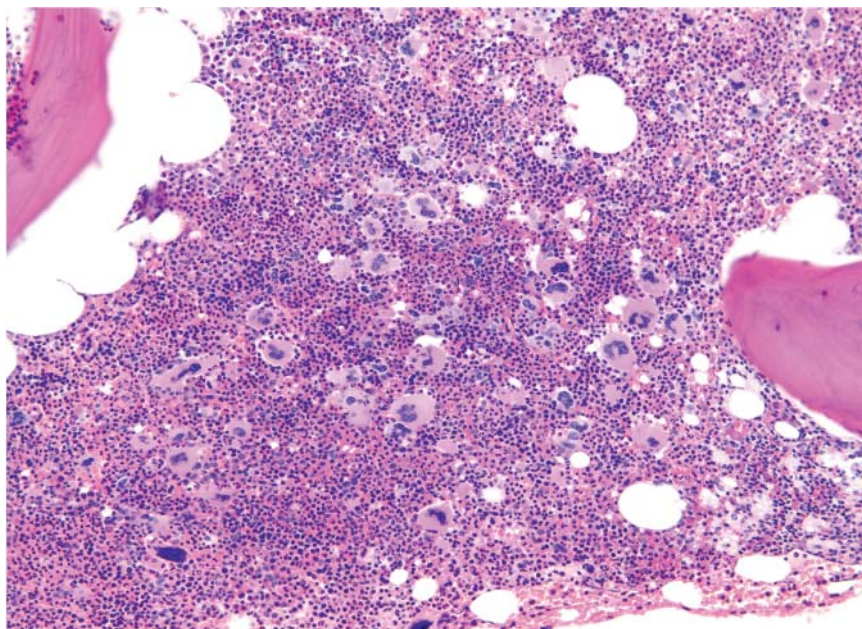
There is a higher incidence of hemorrhage in patients who have extremely high platelet counts ( $>1000 \times 10^9/L$ ). Part of the reason is the absorption of larger VWF multimers onto platelet membranes and subsequent removal from the circulation, resulting in acquired von Willebrand syndrome (Figs 7-5A and B).

### **Chronic Eosinophilic Leukemia, Not Otherwise Specified (CEL, NOS)**

Chronic eosinophilic leukemia is a BCR-ABL negative, clonal myeloproliferative neoplasm with a striking eosinophilia in peripheral blood and in the bone marrow.



**Fig. 7-5A: Essential thrombocythemia.** Marked increase in platelets. Platelet size ranges from very small to giant-sized platelets (Peripheral blood smear).



**Fig. 7-5B: Essential thrombocythemia.** Markedly hypercellular marrow with increased myelopoiesis and megakaryocytosis (Bone marrow section).

**WHO diagnostic criteria of chronic eosinophilic leukemia (myeloproliferative neoplasm with prominent eosinophilia)**

1. Persistent eosinophilia (eosinophils  $>1500/\mu\text{l}$  or  $1.5 \times 10^9/\text{L}$ ).
2. No Philadelphia chromosome or BCR/ABL fusion gene or other MPN (PV, ET, PMF) or MDS/MPN (CMML, aCML).
3. No t(5;12)(q31-35;p13) or other rearrangement of PDGFRB.
4. No FIP1L1- PDGFRA fusion gene or other rearrangement of PDGFRA.
5. No FIP1L1 rearrangement.
6. The blast cell count in the peripheral blood and in the bone marrow is less than 20% and there is no inv(16)(p13q22) or t(16;16)(p13;q22) or other feature diagnostic of AML.
7. There is a clonal cytogenetic or molecular genetic abnormality, or blast cells are more than 2% in the peripheral blood or more than 5% in the bone marrow.

If patient has eosinophilia but these criteria are not met, the differential diagnosis may be reactive eosinophilia, idiopathic hypereosinophilia or idiopathic hypereosinophilic syndrome.

Idiopathic hypereosinophilic syndrome is a diagnosis of exclusion, with increased eosinophils  $>1.5 \times 10^9/\text{L}$  at least for 6 months, and no increase in blasts.

## **Mastocytosis**

Mastocytosis is a pathologic accumulation of mast cells, it may occur at any age. The skin is the most commonly involved organ system, followed by bone marrow, liver, spleen, lymph nodes, and gastrointestinal tract. Clinical presentation includes pruritus, flushing, urtication, abdominal pain, nausea, vomiting, diarrhea, musculoskeletal pain, vascular instability, headache, and neuropsychiatric difficulties.

## **WHO Classification of Mastocytosis**

1. Cutaneous mastocytosis (CM).
2. Indolent systemic mastocytosis (ISM).
3. Systemic mastocytosis with associated clonal hematological non-mast-cell lineage disease (SM-AHNMD).
4. Aggressive systemic mastocytosis (ASM).
5. Mast cell leukemia (MCL).
6. Mast cell sarcoma.
7. Extracutaneous mastocytoma.

**WHO diagnostic criteria for cutaneous and systemic mastocytosis**

1. **Cutaneous mastocytosis:** Skin only.
2. **Systemic mastocytosis:** Mast cell proliferation in tissue other than skin, with the major criteria and one minor criteria or at least three minor criteria present.

1. **Major** (criteria for both): Multifocal, dense infiltrates of mast cells (**≥ 15 mast cells in aggregates**) detected in sections of bone marrow or other extracutaneous organs.

2. **Minor**

- a. In biopsy sections of bone marrow or other extracutaneous organs, >25% of the mast cells in the infiltrate are spindle-shaped or have atypical morphology, or of all mast cells in bone marrow aspirate smear, >25% are immature or atypical.
- b. Detection of an activating point mutation at codon **816 of c-kit** in the peripheral blood, bone marrow or other extracutaneous organs.
- c. Mast cell in peripheral blood, bone marrow or other extracutaneous organs express **CD2**, and/or **CD25** in addition to normal mast cell markers (CD45, CD33, CD117 and CD68).
4. Serum total **tryptase** persistently exceeding 20 ng/ml (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid).

**Imatinib**, a tyrosine kinase inhibitor, has been used in treatment of CML (BCR/ABL), GIST (*c-kit*) and Codon 816 negative *c-kit* or myeloid neoplasm rearrangement of PDGFA. **Imatinib will not work on *c-kit* with codon 816 mutation** (Figs 7-6A to G).

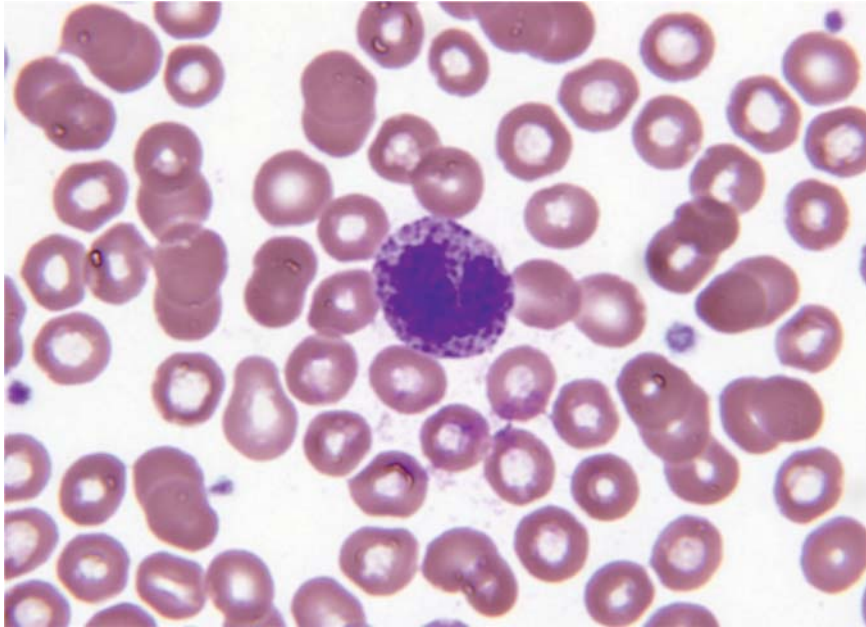
## **Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of PDGFA, PDGFRB or FGFR1**

They constitute three rare specific disease groups, all resulting from an aberrant tyrosine kinase. The aberrant tyrosine kinase activity makes these diseases responsive to tyrosine kinase inhibitors. Eosinophilia is characteristic but variable. The cell of origin is a mutated pluripotent (myeloid-lymphoid) stem cell (Table 7-3).

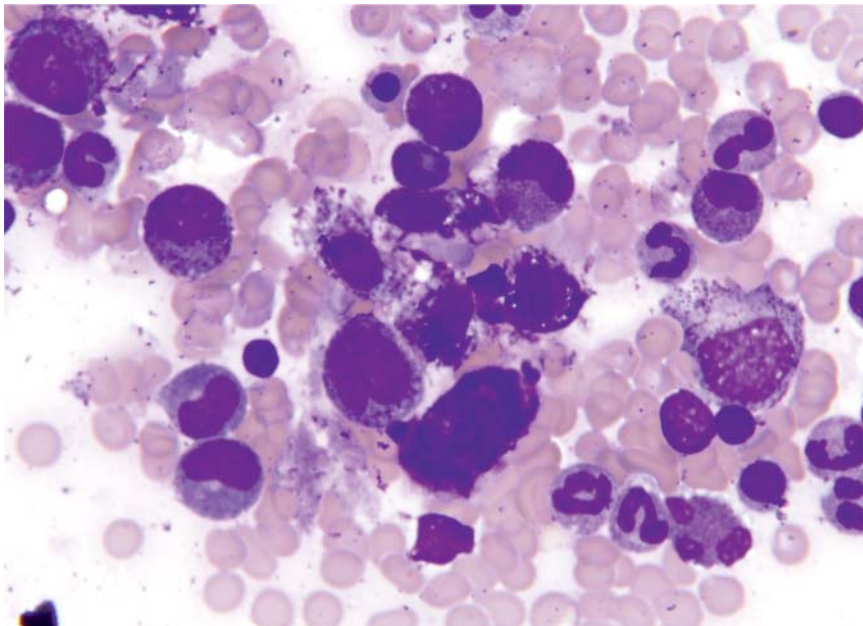
### **Myeloid and Lymphoid Neoplasms with PDGFA Rearrangement**

The most common MPN associated with PDGFA rearrangement is that associated with FIP1L1-PDGFA fusion gene, a **cryptic** deletion at 4q12.



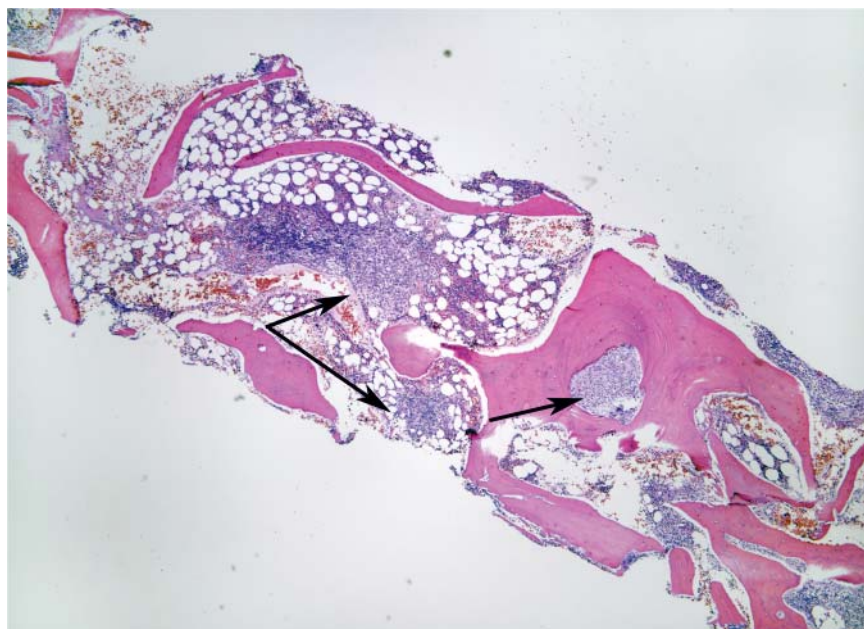


**Fig. 7-6A: Systemic mastocytosis.** Showing a circulating mast cell (Peripheral blood smear).

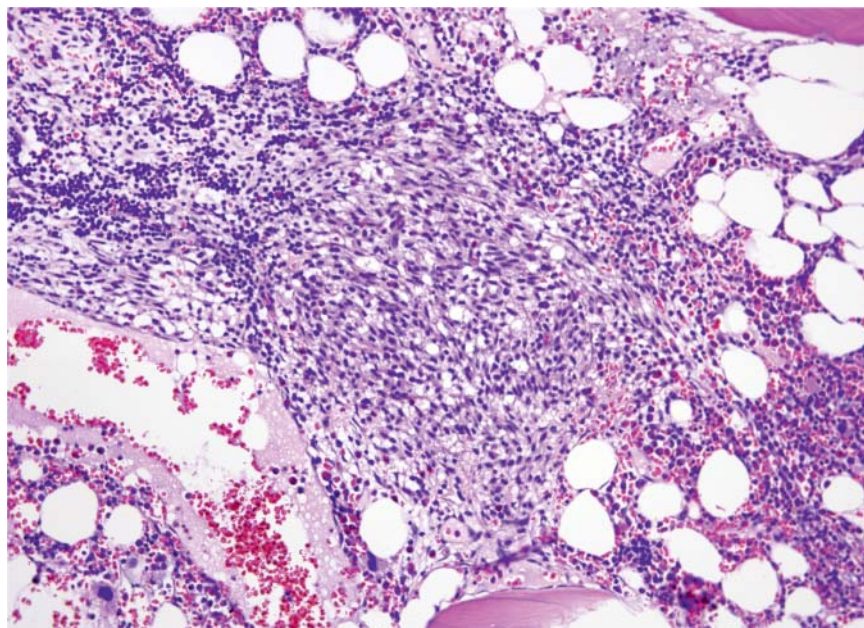


**Fig. 7-6B: Systemic mastocytosis.** Numerous mast cells containing large dark blue to purple granules that usually obscure the nucleus (Bone marrow aspirate).

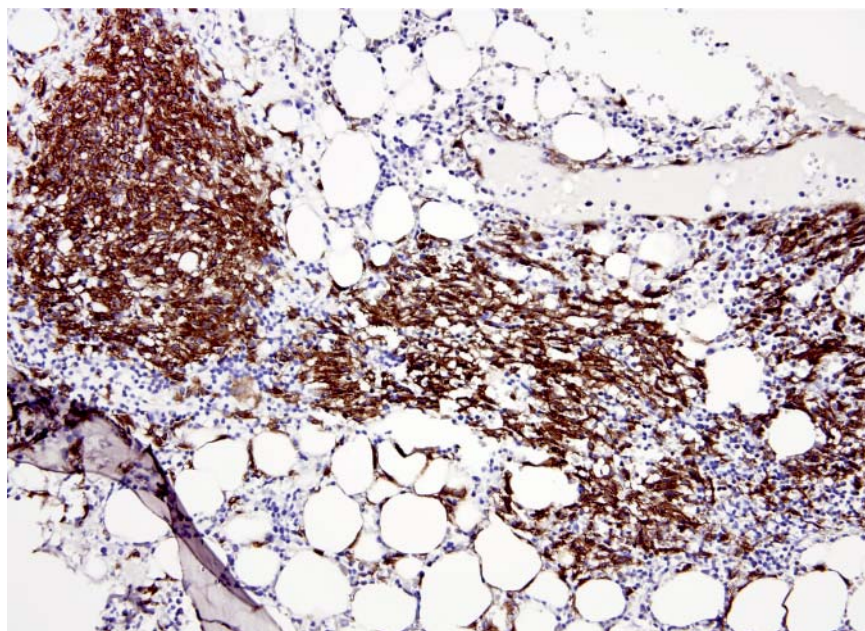




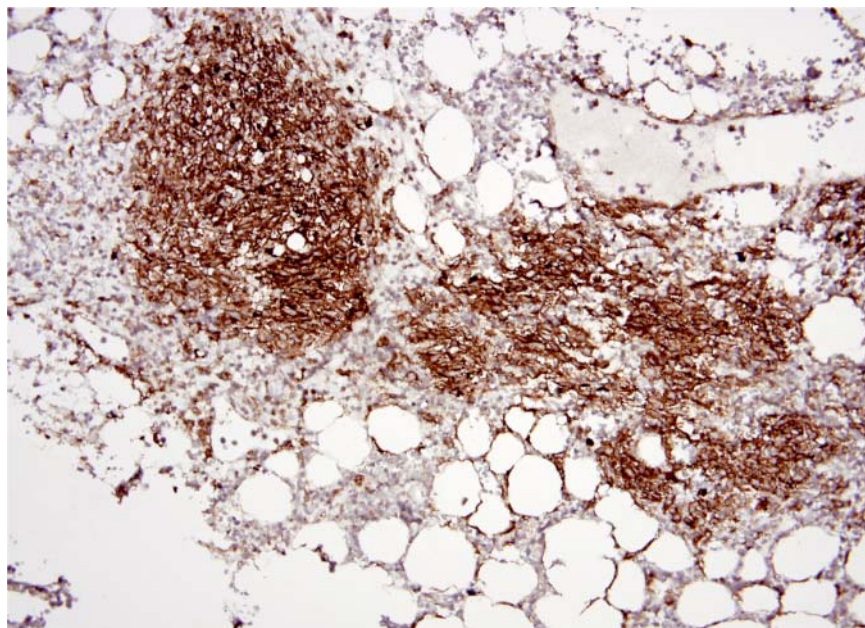
**Fig. 7-6C: Systemic mastocytosis.** Aggregates of mast cells (arrows) present in the bone marrow. Mast cells may be increased in other clonal lymphoid disorders, such as Waldenström macroglobulinemia, and SLL/CLL (Bone marrow section).



**Fig. 7-6D: Systemic mastocytosis.** At higher magnification, these mast cells form spindle cell aggregates, and are frequently accompanied by eosinophils (Bone marrow section).

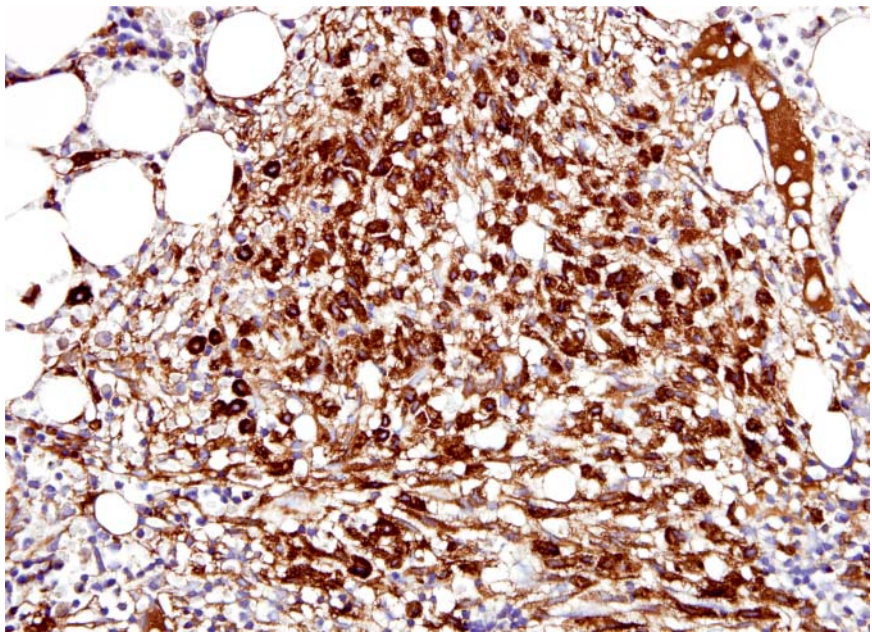


**Fig. 7-6E**



**Fig. 7-6F**





**Fig. 7-6G**

**Figs 7-6E to G: Systemic mastocytosis.** Immunohistochemical studies for CD2, CD25 (E), CD117 (F), and tryptase (G) can be used to identify neoplastic mast cells. These neoplastic mast cells are usually CD2, and/or CD25, CD117, and tryptase positive (Bone marrow section).

**TABLE  
7-3**

**Molecular variants of ETV6-PDGFRB with eosinophilia**

Diagnosis	Fusion protein	Translocation
CEL	WDR48-PDGFRB	t(1;3;5)(p36;p21;q33)
	GPIAP1-PDGFRB	der(1)t(1;5)(p34;q33) der(5)t(1;5)(p34;q15) der(11)ins(11;5)(p12;q15q33)
	TPM3-PDGFRB	t(1;5)(q21;q33)
	GIT2-PDGFRB	t(5;12)(q31-33;q24)
CMML	HIP1-PDGFRB	t(5;7)(q33;q11.2)
	KIAA1509-PDGFRB	t(5;14)(q33;q32)
MPD/MDS	PDE4DIP-PDGFRB	t(1;5)(q21;q33)
aCML	CCDC6-PDGFRB	t(5;10)(q33;q12)
	TP53BP1-PDGFRB	t(5;15)(q33;q22)

Conventional cytogenetic analysis is normal. Clinical presentation is generally chronic eosinophilic leukemia (CEL) but can be present as AML, T lymphoblastic leukemia/lymphoma or both simultaneously.

### ***Myeloid Neoplasms with PDGFB Rearrangement***

A distinctive type of myeloid neoplasm which occurs in association with rearrangement of PDGFRB at 5q31-33, commonly t(5;12)(q31-33;p12) with formation of ETV6-PDGFRB. Clinical presentation can be CMML, aCML, MPN with eosinophilia or CEL.

### ***Myeloid and Lymphoid Neoplasms with FGFR1 Abnormalities***

They are heterogenous. The clinical presentation may be as CEL, AML, and T lymphoblastic leukemia/lymphoma or less often, precursor B lymphoblastic leukemia/lymphoma. Lymphoblastic lymphoma appears to be more common among patients with t(8;13) translocation. Basophilia may present in the patients who have BCR-FGFG1 fusion gene.

### **Myeloproliferative Neoplasm, Unclassifiable**

Myeloproliferative neoplasm unclassifiable (MPN, U) should be applied only to cases that have definite clinical, laboratory and morphological features of an MPN but fail to meet the criteria for any specific MPN entities, or that present features that overlap two or more of the MPN categories. Most cases of MPN, U will fall into one of three groups:

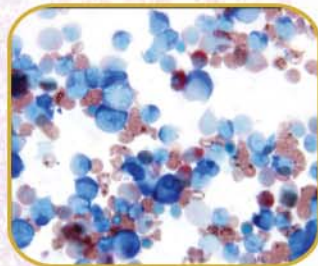
1. Early stages of PV, PMF, or ET in which characteristic features are not yet fully developed.
2. Advanced stage MPN, in which pronounced myelofibrosis, osteosclerosis, or transformation to a more aggressive stage obscures the underlying disorder.
3. Patients with convincing evidence of an MPN in whom a coexisting neoplastic or inflammatory disorder obscures some of the diagnostic and/or morphological features.



CHAPTER

8

# Acute Myeloid Leukemia





**The diagnosis of acute myeloid leukemia (AML) requires a blast count of  $\geq 20\%$  in the peripheral blood or in the bone marrow.**

AML with t(8;21), inv(16)(p13;q22) or t(16;16), or (15;17) are classified as AML regardless of the blast count.

It is not yet clear if cases with t(9;11), t(6;9), inv(3) or t(3;3) or t(1;22) should be categorized as AML when the blast cell count is less than 20%. Close clinical follow up is recommended (WHO 2008).

## ***WHO Classification and Prognosis***

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### **WHO Classification of AML**

#### **1. AML with recurrent genetic abnormalities**

- a. AML with t(8;21)(q22;q22) RUNX1 (AML1 or CBFA)-RUNX1T1 (ETO) fusion (FAB subtype M2).
- b. AML with eosinophils inv(16)(p13;q22) or t(16;16)(p13;q22) CBFb-MYH11 fusion (FAB subtype M4e).
- c. Acute promyelocytic leukemia (APL) with (15;17)(q22;q12) PML-RARA fusion and its variant (FAB subtype M3).
- d. AML with t(9;11)(q22;q23) MLLT3-MLL and its variant MLL translocation.
- e. AML with t(6;9)(p23;q34) DEK-NUP214.
- f. AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) RPN1-EVI1.
- g. AML (megakaryoblastic) with t(1;22)(p13;q13) RBM15-MKL1.
- h. AML with gene mutations.

#### **2. AML with myelodysplasia related changes.**

#### **3. Therapy-related myeloid neoplasms.**

#### **4. AML not otherwise categorized.**

- a. AML minimally differentiated (FAB subtype M0).
- b. AML without maturation (FAB subtype M1).
- c. AML with maturation [FAB subtype M2 except t(8;21)].
- d. Acute myelomonocytic leukemia (FAB subtype M4).
- e. Acute monoblastic and monocytic leukemia (FAB subtype M5).
- f. Acute erythroid leukemia (FAB subtype M6).
- g. Acute megakaryoblastic leukemia (FAB subtype M7).
- h. Acute basophilic leukemia.
- i. Acute panmyelosis with myelofibrosis.
- j. Myeloid sarcoma.

#### **5. Acute leukemia of ambiguous lineage**

- a. Undifferentiated acute leukemia.

- b. Bilinear acute leukemia: two populations, mixed lymphoid and myeloid blasts.
- c. Biphenotypic acute leukemia: one population, express both lymphoid and myeloid markers on the same blast population.

### Cytochemical Stains Used in Lineage Identification (Table 8-1)

1. Myeloperoxidase (**MPO**): usually myeloid lineage positive.
2. Sudan Black B (**SBB**): usually myeloid lineage positive
3. Non-specific esterase (**NSE**): Monocytes positive (gray-black color)
4. Chloroacetate esterase (**CAE**): Myeloid lineage positive (reddish color)
5. Periodic acid-Schiff (**PAS**): typical block stain pattern in lymphoblasts. Erythroleukemia, megakaryoblastic or monocytic leukemia can be positive.

**TABLE 8-1**

**Cytochemical stain pattern of acute myeloid, monocytic and acute lymphoblastic leukemia**

	MPO	SBB	CAE	NSE	PAS
Myeloid	+	+	+	–	–
Monocytic	–	–	–	+	–/+
ALL	–	–	–	–	+

### Prognosis

AML remission, survival and cure rates are most dependent on the patient's age, cytogenetic risk category and expression of multiple drug resistance (MDR) genes in leukemic cells (Table 8-2).

### Detection of Minimal Residual Disease

FISH, flow cytometry and PCR are used for the detection of minimal residual disease (undetectable by light microscopic examination).

#### **Detection of Inversion 16: PCR or FISH**

After complete chemotherapy, patients with 10 copies or more of CBF-/MYH11 fusion gene have a shorter remission duration and higher risk for relapse.

#### **Detection of t(8;21): PCR or FISH**

This translocation fuses the RUNX1 gene on chromosome 21p to ETO on chromosome 8p to produce a fusion gene. The fusion gene presents in

**TABLE  
8-2****Prognosis and cytogenetics in AML**

favorable	inv(16) or t(16;16)* t(8;21)* Single miscellaneous defect Normal karyotype with CEBPA mutation* Normal karyotype with NPM1 but no FLT3-ITD mutation*
intermediate	t(15;17) +8 t(6;9) t(9;11) in children Normal*
unfavorable	-5, -7, -X, -Y, +8, 5q-, 7q-, +21 t(11q23) inv(3q) Normal karyotype with FLT3-ITD mutation inv(16) or t(8;21) with c-KIT mutations Complex karyotype.

Note:

- \* Normal karyotype with FLT3-ITD (FMS-related tyrosine kinase internal tandem duplications) mutations is associated with an adverse outcome.
- \* Normal karyotype with NPM1 (Nucleophosmin) mutation but a negative FLT3-ITD mutation is associated with a favorable outcome.
- \* Normal karyotype with NPM1 and FLT3-ITD mutations are associated with a poorer prognosis but have an improved prognosis compared to NPM1 negative and FLT3-ITD positive AML.
- \* Normal karyotype with CEBPA (CCAAT/enhancer binding protein-a) is associated with a favorable outcome similar to AML with inv(16) or t(8;21).
- \* inv(16) or t(8;21) with c-KIT mutations appears to have an adverse prognosis.

majority of remission patients, but not in patients who received allogeneic hematopoietic stem cell transplantation.

### ***Detection of t(15;17): PCR or FISH***

Unlike AML with the fusion transcript t(8;21), in APL the t(15;17) fusion transcript usually disappears after intensive therapy, thus achieving a molecular remission (negative RT-PCR).

### ***Detection of NPM-1 and FLT-3 Mutations: PCR***

In AML patients who have normal cytogenetics, mutational status of NPM1, FLT3, CEPBA, MLL, and RAS may have implications for treatment outcomes and prognosis.

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***AML with Recurrent Genetic Abnormalities***

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**AML with t(8;21)(q22;q22)**

1. Favorable prognosis in adult.
2. Approximately 5-10% cases of all AML.
3. Classify as AML regardless of the blast count.
4. Predominantly in younger patients.
5. RUNX1 (AML1 or CBFA)-RUNX1T1 (ETO) fusion protein.
6. Blasts are positive for CD34, HLA-DR, MPO, CD15, and CD56 and show weakly expression of CD33.

**AML with Eosinophils inv(16)(p13;q22) or t(16;16)(p13;q22)**

1. Favorable prognosis in adult.
2. Approximately 8% cases of all AML.
3. Classify as AML regardless of the blast count.
4. Acute myelomonocytic leukemia with dysplastic eosinophils in bone marrow.
5. CBFβ-MYH11 fusion protein.
6. Lymphadenopathy and hepatomegaly common.
7. Risk of extramedullary myeloid sarcoma is 50% higher than other AML.
8. Blast expression of CD34, CD117, myeloid markers (CD13, CD33, CD15, CD65, MPO), and monocyte markers (CD4, CD11b, CD11c, CD14, CD36, CD64).

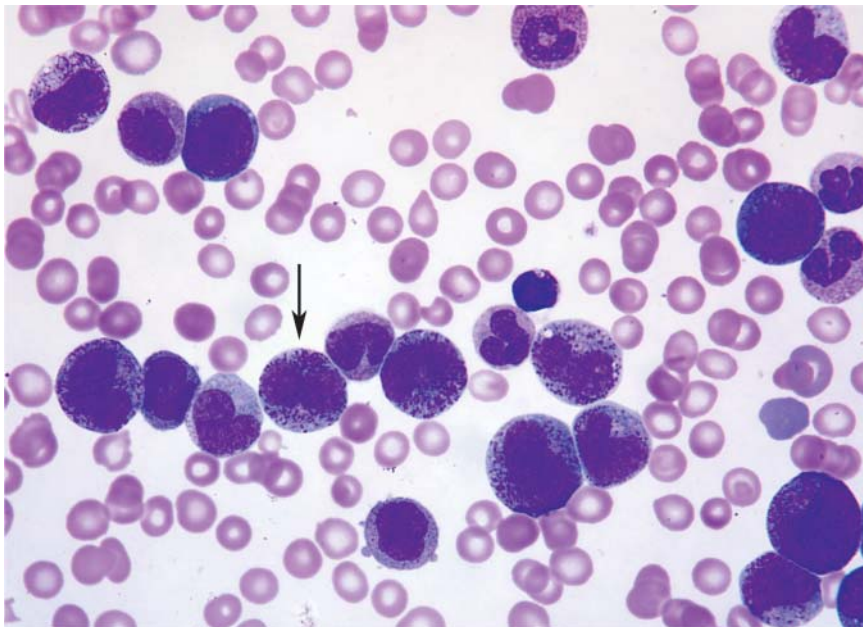
**Acute Promyelocytic Leukemia (APL) with t(15;17)(q22;q12)**

1. Intermediate prognosis.
2. Approximately 8-10% cases of all AML.
3. Classify as AML regardless of the blast count.
4. PML-RARA fusion protein.
5. Responds to all-trans retinoic acid (ATRA) treatment.
6. Commonly associated with DIC and bleeding (STAT FISH should be performed if clinically suspect APL).
7. 10% cases are the hypogranular variant.
8. Leukemic cells show bright expression of CD33, but CD34 and HLA-DR are negative.

9. The leukemic cells contain numerous red to purple cytoplasmic granules, bundles of Auer rods (“faggot cells”) and bilobed or kidney-shaped nuclei.
10. The hypogranular (microgranular) variant leukemic cells contain paucity or absence of cytoplasmic granules.
11. Other cytogenetic variants t(V;17) do not respond to ATRA therapy and is more aggressive (Figs 8-1A to D).

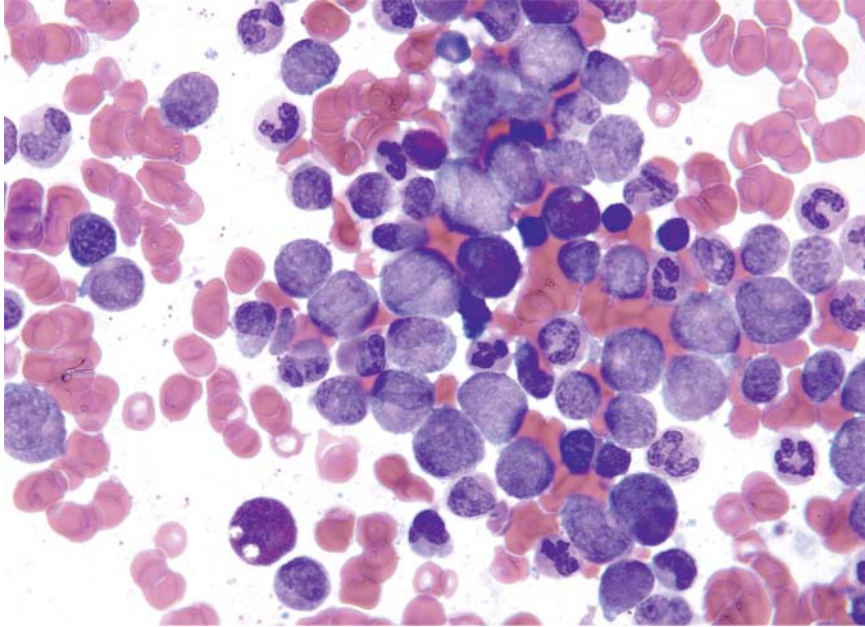
### AML with t(9;11)(q22;q23) MLLT3-MLL

1. Associated with monocytic features, commonly seen in children.
2. Approximately 9-12% cases of pediatric AML and 2% cases of adult AML.
3. May present as extramedullary sarcoma (gingiva, skin) and DIC.
4. Blasts show strong expression of CD33, CD65, CD4, HLA-DR, and low expression of CD13, CD14, and CD34.
5. 11q23 (MLL) translocation is also associated with
  - Topoisomerase II inhibitor therapy related AML

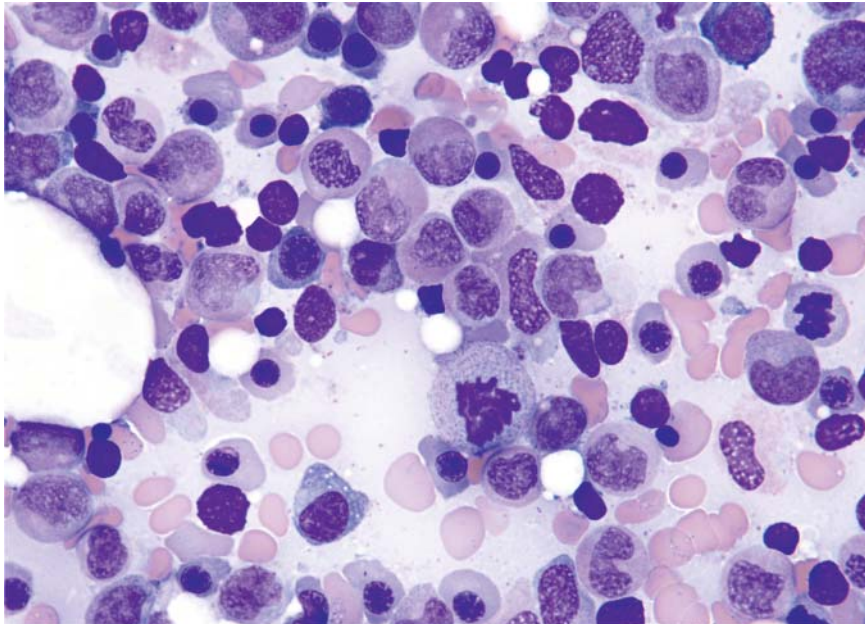


**Fig. 8-1A: Acute promyelocytic leukemia.** Leukemic promyelocytes with convoluted nuclei (dumbbell shape)(arrow) and granules in the cytoplasm (Bone marrow aspirate).

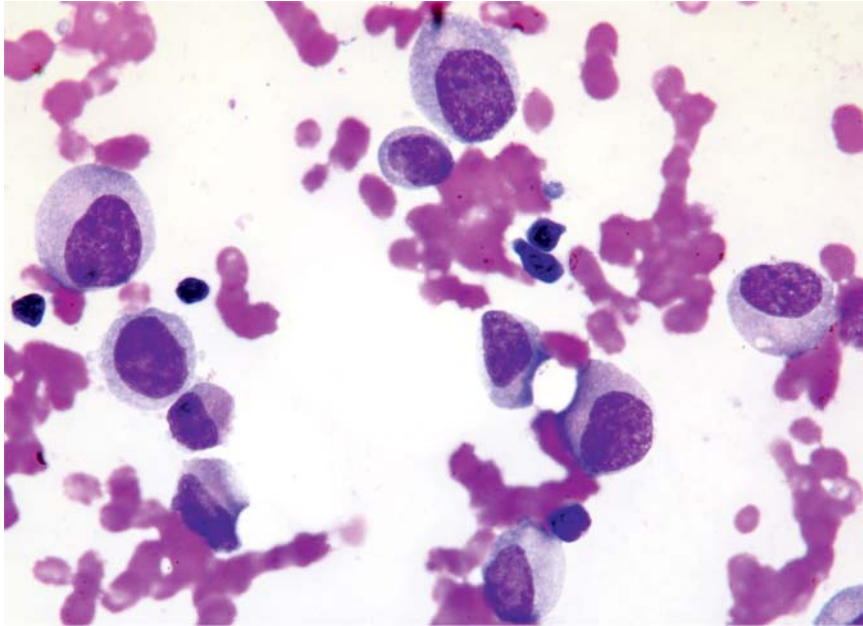




**Fig. 8-1B: Acute promyelocytic leukemia.** Markedly increased promyelocytes, with occasional cells containing numerous cytoplasmic reddish-purple granules or Auer rods (faggot cells) (Bone marrow aspirate).



**Fig. 8-1C: Acute promyelocytic leukemia hypogranular variant.** The leukemic promyelocytes do not contain numerous cytoplasmic reddish-purple granules (Bone marrow aspirate).



**Fig. 8-1D: Acute promyelocytic leukemia hypogranular variant.** The leukemic promyelocytes do not contain numerous cytoplasmic reddish-purple granules (Bone marrow aspirate).

- Biphenotypic leukemia
  - Acute monoblastic and monocytic leukemia
  - ALL
6. The prognosis of AML with 11q23 (MLL) abnormalities are poor, except for monocytic leukemia with t(9;11) in children, which has an intermediate survival.

### **AML with t(6;9)(p23;q34) DEK-NUP214**

1. Poor prognosis.
2. Often associated with basophilia and multilineage dysplasia.
3. Approximately 0.7-1.8% cases of all AML.
4. Blast expression of MPO, CD33, CD34, CD38, and HLA-DR.
5. FLT3-ITD mutation is common, and present in 69% of pediatric cases and 78% of adult cases.

### **AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) RPN1-EVI1**

1. Poor prognosis.
2. Approximately 1-2% cases of all AML.

3. Often associated with increased peripheral blood platelet counts, increased atypical megakaryocytes in the bone marrow, and multilineage dysplasia.
4. Blast expression of CD13, CD33, CD34, CD38, and HLA-DR.

### **AML (Megakaryoblastic) with t(1;22)(p13;q13) RBM15-MKL1**

1. Generally shows megakaryocyte lineage.
2. Clinical presentation similar to acute megakaryocyte leukemia of AML, NOS.

### **AML with Gene Mutations**

In addition to translocations and inversions, specific gene mutations may occur in AML and relate to prognosis.

1. AML with NPM1 mutation: usually present without history of MDS or MPN. Patients often exhibit anemia and thrombocytopenia. There is strong association with acute myelomonocytic and acute monocytic leukemia. AML with NPM1 mutation but a negative FLT3-ITD mutation is associated with a favorable outcome.
2. AML with FLT3-ITD (FMS-related tyrosine kinase internal tandem duplications) mutations is associated with an adverse outcome.
3. AML with NPM1 and FLT3-ITD mutations are associated with a poor prognosis but have an improved prognosis compared to NPM1 negative and FLT3-ITD positive AML.
4. AML with CEBPA (CCAAT/enhancer binding protein- $\alpha$ ) is associated with a favorable outcome similar to AML with inv(16) or t(8;21).
5. AML with inv(16) or t(8;21) and c-KIT mutations appear to have an adverse prognosis.

### ***AML with Myelodysplasia Related Changes***

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The prognosis is poor with low rate of complete remission.

#### **1. Diagnostic Criteria (WHO 2008):**

≥ 20% blasts in peripheral blood or bone marrow

*and*

any of the following:

1. Previous history of MDS.
2. MDS related cytogenetic abnormality (see list below).

3. Multilineage dysplasia  
and

absence of both:

1. Prior cytotoxic therapy for an unrelated disease.
2. Recurring cytogenetic abnormality as described in AML with recurrent genetic abnormalities.

**2. Cytogenetic abnormalities sufficient to diagnose AML with MDS related features when  $\geq 20\%$  blasts in peripheral blood or bone marrow are present:**

1. -7/del(7q)
2. -5/del(5q)
3. i(17q)/t(17p)
4. -13/del(13q)
5. del(11q)
6. del(12p)/t(12p)
7. del(9q)
8. idic(X)(q13)
9. t(11;16)(q23;p13.3)\*
10. t(3;21)(q26.2;q22.1)\*
11. t(1;3)(p36.3;q21.1)
12. t(2;11)(p21;q23)\*
13. t(5;12)(q33;p12)
14. t(5;7)(q33;q11.2)
15. t(5;17)(q33;p13)
16. t(5;10)(q33;q21)
17. t(3;5)(q25;q34)

\* Therapy-related AML must be excluded.

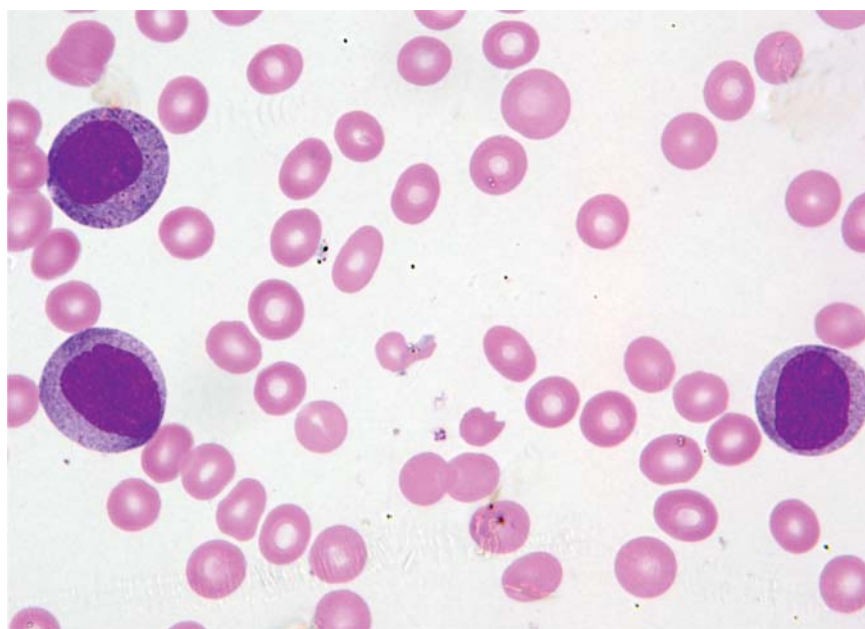
### ***Therapy-related Myeloid Neoplasms (t-AML, t-MDS, t-MDS/MPN)***

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1. Overall prognosis is poor.
2. Alkylating drug therapy-related MDS is commonly associated with multilineage dysplasia and chromosome 5 and/or chromosome 7 abnormalities.
3. Topoisomerase II inhibitor therapy is often associated with monocytic or myelomonocytic leukemia and chromosome 11q23 abnormalities.

***AML Not Otherwise Categorized*****AML with Minimal Differentiation (FAB Subtype M0)**

1. Less than 5% cases of all AML.
2. Poor prognosis.
3. Less than 3% of blasts are positive for cytochemical MPO stain.
4. Blast expression of CD34, HLA-DR and one or more myeloid markers (CD13, CD33, and CD117).
5. Aberrant expression of lymphoid markers (CD2, CD4, CD7, and CD19) may be present.
6. TdT is positive in 30-50% of the cases (Fig. 8-2).



**Fig. 8-2: Acute myeloid leukemia, minimally differentiated**  
(Peripheral blood smear).

**AML without Maturation (FAB Subtype M1)**

1. Approximately 10% cases of all AML.
2.  $\geq 3\%$  of blasts are positive for cytochemical MPO or Sudan black B stains.
3. Myeloblasts must be  $\geq 90\%$  of the non-erythroid cells in the bone marrow.
4. Blast expression of CD34, HLA-DR and one or more myeloid markers (CD13, CD33, CD117).



### **AML with Maturation (FAB Subtype M2)**

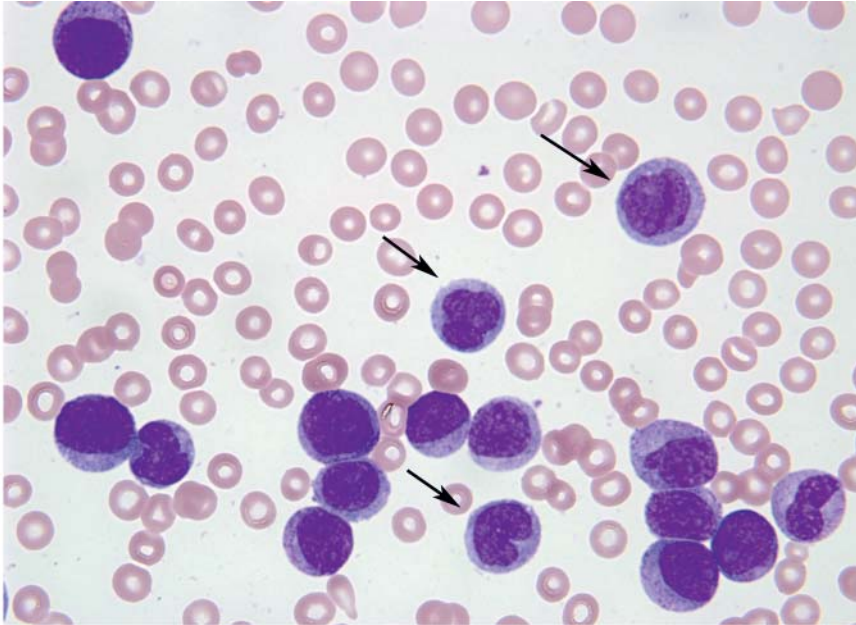
1. Most common subtype, approximately 30-45% cases of AML.
2. Evidence of maturation beyond myeloblasts.
3. Myeloblasts account for 20-89% of non-erythroid cells in the bone marrow.
4. >10% of the granulocytes mature to neutrophils.
5. Monocytes are less than 20%.
6. Blast expression of CD34, HLA-DR and one or more myeloid markers (CD13, CD33, CD117).

### **Acute Myelomonocytic Leukemia (AMML, FAB Subtype M4)**

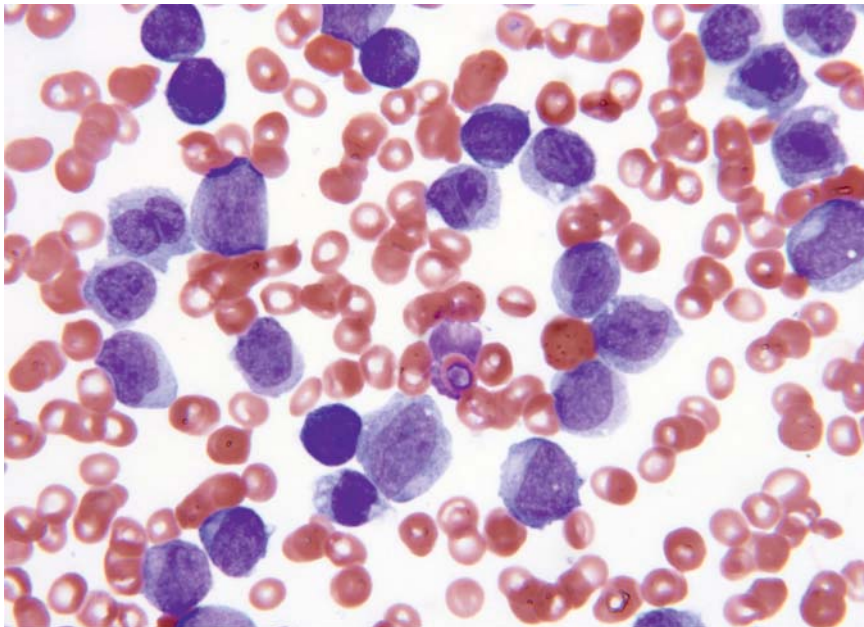
1. Approximately 15-20% cases of AML.
2. The sum of myeloblasts, monoblasts, and promonocytes must be  $\geq 20\%$  of the non-erythroid cells in the bone marrow.
3. Cytochemical stains demonstrate 20-79% of the bone marrow cells are of monocyte lineage.
4. If monocytes are <20% in bone marrow but  $>5 \times 10^9/L$  in the peripheral blood, the diagnosis is still AMML, not AML.
5. Blast expression of myeloid and monocyte markers, some blasts show aberrant expression of CD7 and HLA-DR (Figs 8-3A and B).

### **Acute Monoblastic and Monocytic Leukemia (FAB Subtype M5)**

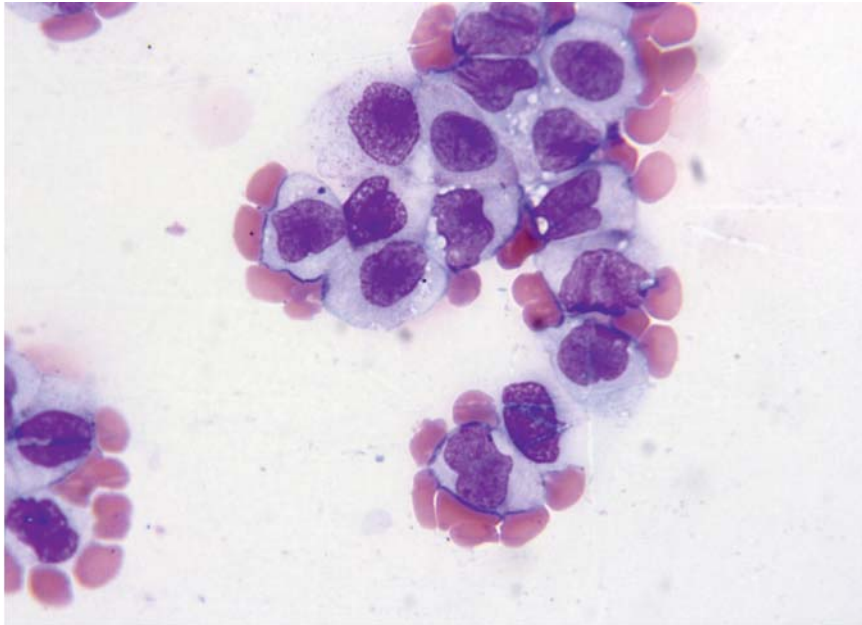
1. Approximately 5-10% cases of AML.
2. Commonly associated with bleeding disorder, extramedullary masses, skin, gingiva, and CNS involvement.
3. The sum of monoblasts, promonocytes or monocytes must be  $\geq 80\%$  of the non-erythroid cells in the bone marrow.
4. Acute monoblastic leukemia (FAB subtype 5a, poorly differentiated):  $\geq 80\%$  of the monocytic cells are monoblasts.
5. Acute monocytic leukemia (FAB subtype 5b, differentiated): <80% monocytes are monoblasts (the majority of the monocytic cells are promonocytes).
6. Blasts expression of monocyte associated markers (at least two of CD4, CD11b, CD11c, CD14, CD64, CD68, and lysozyme) and myeloid markers (CD13, CD33, CD117) (Figs 8-4A to D).



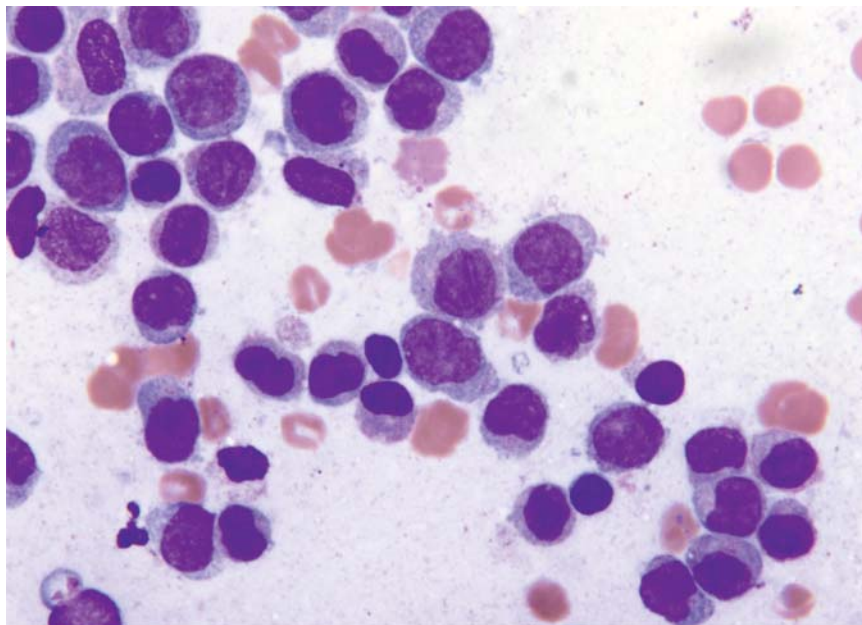
**Fig. 8-3A: Acute myelomonocytic leukemia.** Some of the blasts show irregular and folded nuclear configurations (monoblast, arrows) (Peripheral blood smear).



**Fig. 8-3B: Acute myelomonocytic leukemia.** Myeloid blasts, monocyctic blasts, and promonocytes (Peripheral blood smear).

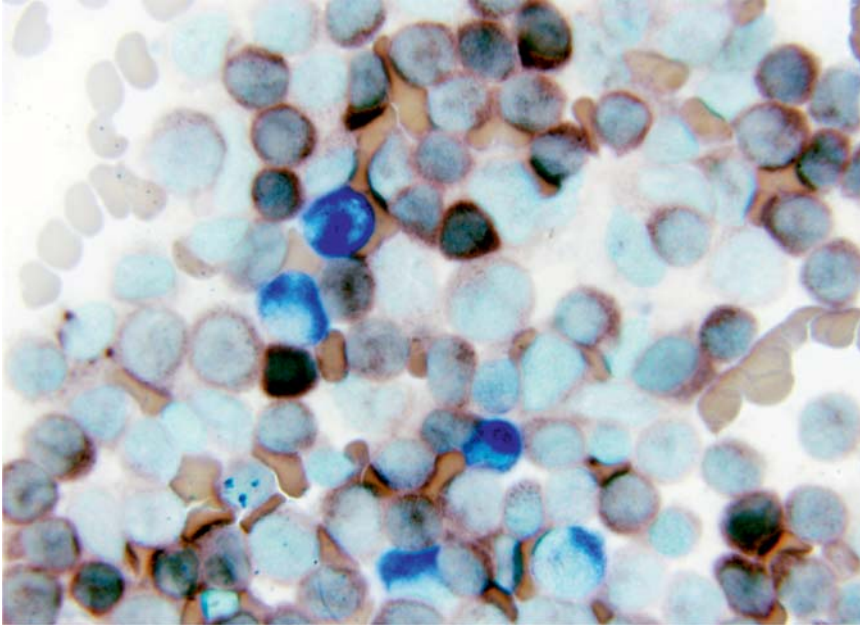


**Fig. 8-4A: Acute monoblastic and monocytic leukemia.** Monoblasts and promonocytes (Peripheral blood smear).

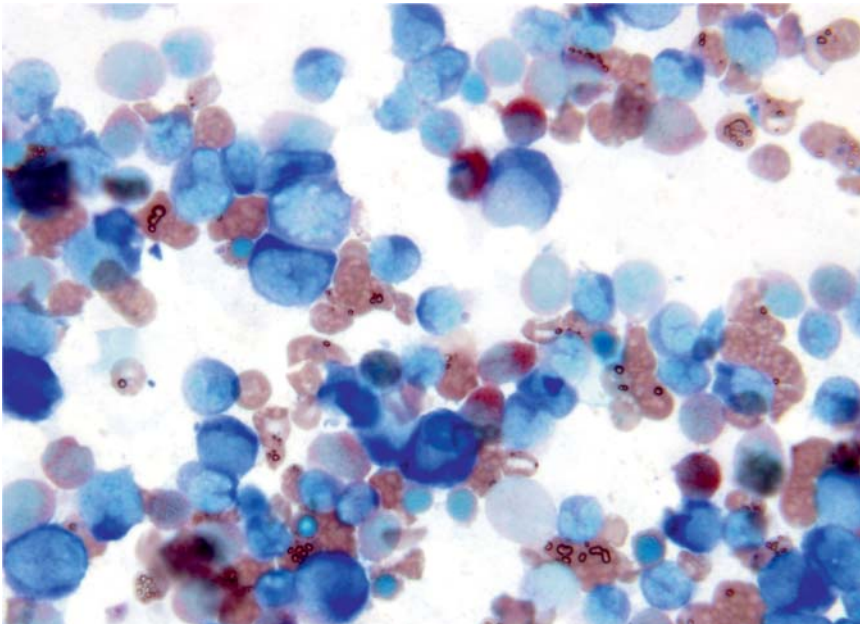


**Fig. 8-4B: Acute monoblastic and monocytic leukemia.** Monoblasts and promonocytes (Bone marrow aspirate).





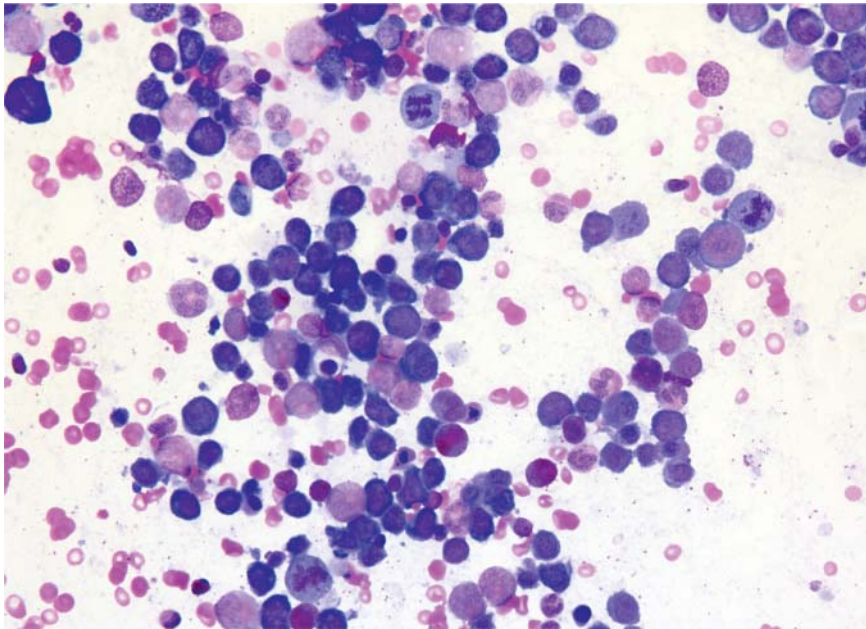
**Fig. 8-4C: Acute monoblastic and monocytic leukemia.** Double esterase stain: Chloroacetate esterase stain (CAE) and non-specific alpha naphthol acetate esterase stain (NSE). Leukemic monoblasts and monocytes. NSE stains monocytes dark gray to black (Bone marrow aspirate).



**Fig. 8-4D: Acute monoblastic and monocytic leukemia.** Double esterase stain. CAE stains myelocytes red/pink (Bone marrow aspirate).

### Acute Erythroid Leukemia (FAB Subtype M6)

1. <5% cases of AML.
2. Erythroid/myeloid leukemia: myeloblasts >20% in non-erythroid series (subtract erythroid series, plasma and lymphoid cells), erythroblast >50% in all nucleated bone marrow cells.
3. Pure erythroid leukemia: >90% of the bone marrow elements are proerythroblasts and early basophilic erythroblasts.
4. Blasts expression of erythroid (hemoglobin A, glycophorin) and myeloid-associated markers. HLA-DR and CD34 are often negative in pure erythroid leukemia.
5. Prognosis is aggressive and poor (Fig. 8-5).



**Fig. 8-5: Acute erythroid leukemia.** Mixture of myeloblasts and erythroblasts with minimal maturation (Bone marrow aspirate).

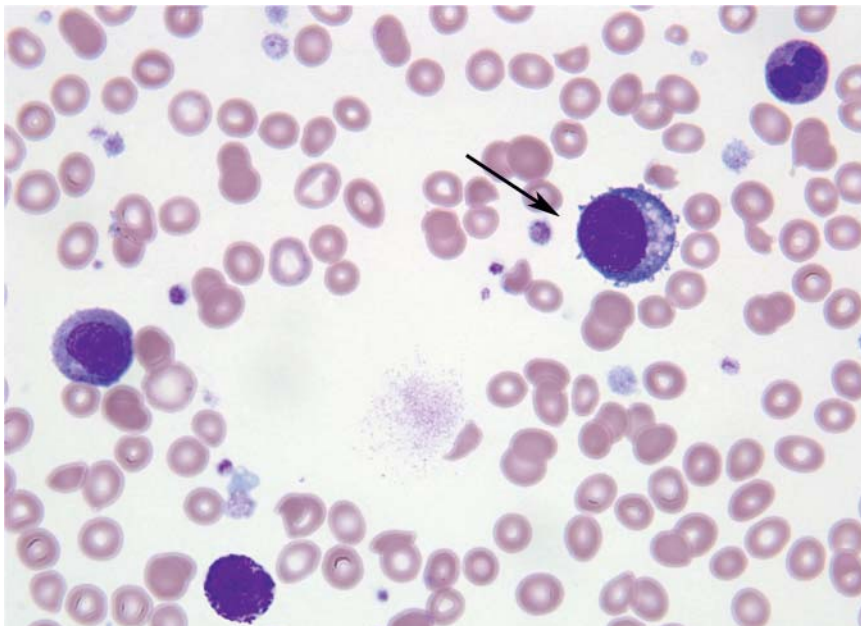
### Acute Megakaryoblastic Leukemia (FAB Subtype M7)

1. Uncommon, <5% cases of AML.
2. Blasts show distinct cytoplasmic blebs (pseudopod formation).
3. Examination by EM shows evidence of a demarcation membrane and bulls-eye  $\alpha$ -granules.
4. Blast expression of megakaryocyte markers (CD41, CD61) and myeloid marker (CD13, CD33). CD34 and HLA-DR are often negative (Fig. 8-6).

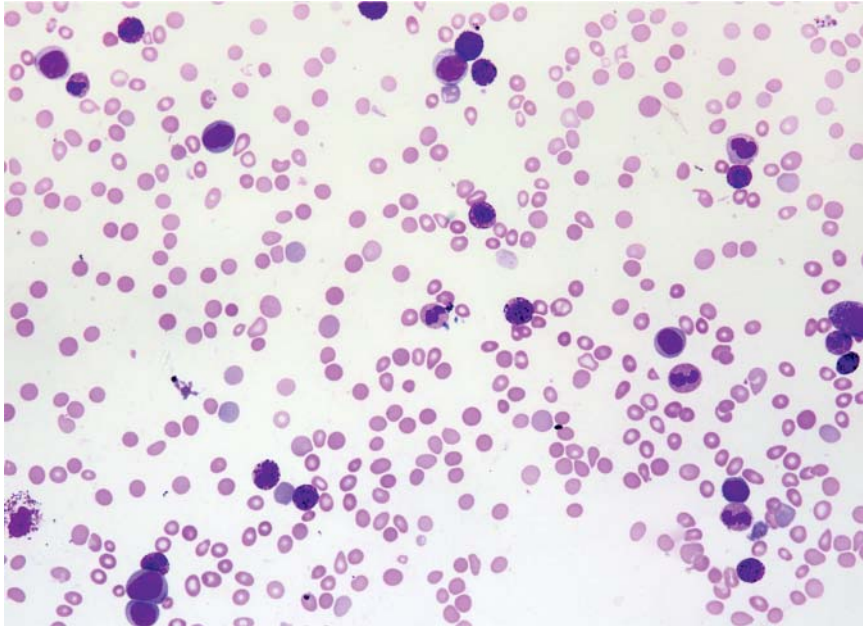


**TABLE  
8-3****Possible diagnoses when erythroid precursors account for  $\geq 50\%$  of the bone marrow nucleated cells**

Diagnosis	% Erythroid precursors	% Blasts	Other findings
AML with myelodysplasia related changes	$\geq 50\%$	$\geq 20\%$	Meets criteria for AML with myelodysplasia related changes
Pure erythroid leukemia	$\geq 80\%$ with minimal maturation	Few myeloblasts may be present	Minimal granulocytes may present
Acute erythroid/myeloid leukemia	$\geq 50\%$	$\geq 20\%$ in peripheral blood or blasts $\geq 20\%$ of all non-erythroid in bone marrow	No evidence of MDS
MDS	$\geq 50\%$	$<20\%$ in peripheral blood or blasts $<20\%$ of all non-erythroid in bone marrow	Evidence of MDS



**Fig. 8-6: Acute megakaryocytic leukemia.** Blast cell with nucleoli and membranous projections (Peripheral blood smear).



**Fig. 8-7: Acute basophilic leukemia.** Blasts without granules and basophils with granules (Peripheral blood smear).

### Acute Basophilic Leukemia

1. Very rare (<1% cases of AML).
2. Blast expression of myeloid markers (CD13, CD33), and other markers CD123, CD203c, and CD11B, may be positive for CD34 and HLA-DR (Fig. 8-7).

### Acute Panmyelosis with Myelofibrosis

1. Very rare and clinically aggressive.
2. Mostly adult cases, children rare.
3. Spleen normal or minimally increased in size (unlike non-Hodgkin lymphomas, myeloproliferative neoplasia and primary myelofibrosis, which has prominent splenomegaly and dysplasia).

### Myeloid Sarcoma

1. A tumor mass consisting of myeloid blast occurring in an anatomical site other than bone marrow.
2. Myelomonocytic leukemia with inv(16), or monoblastic leukemia in children often presents with extramedullary myeloid sarcoma.

### Myeloid Proliferations Related to Down Syndrome (Trisomy 21)

1. 10-100 fold increased risk of leukemia compared to the normal population.
2. 70% cases of AML in patients younger than 4-year-old are acute megakaryoblastic leukemia.
3. Transient abnormal myelopoiesis frequently associated with GATA1 mutation. Transient abnormal myelopoiesis is a unique disorder with Down syndrome, the majority of patients show spontaneous remission within the first three months of life.
4. Trisomy 8 is common in Down syndrome related AML, while monosomy 7 is very rare.
5. Compared to non-Down syndrome patients, AML with GATA1 mutation of younger patients have better response to chemotherapy while older children AML with GATA1 mutation have poor prognosis.

### Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDC)

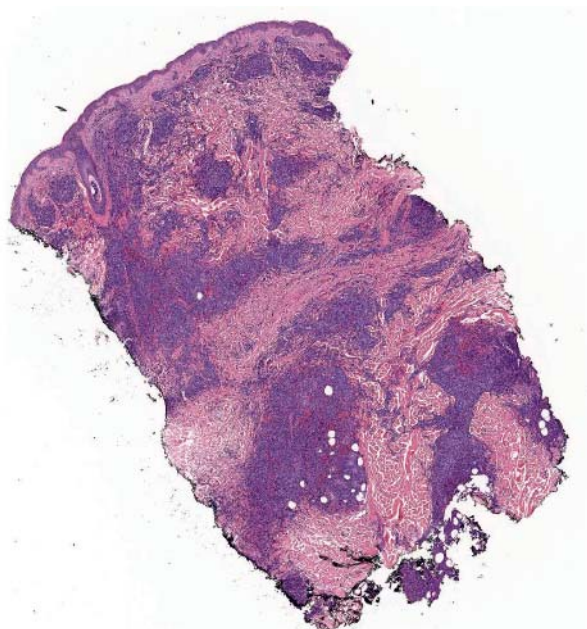
1. Previously named as CD4+/CD56+ hematodermic neoplasm, blastic NK cell leukemia/lymphoma.
2. Disease involves the skin (100% of cases), followed by bone marrow, peripheral blood (60-90%), and lymph nodes (40-50%).
3. The tumor cells express CD4, CD43, CD45RA, CD56, and **plasmacytoid dendritic cell** antigens **CD123**, BDCA-2/CD303, TCL1. CD56 can be negative in rare cases.
4. TCR and IgH gene rearrangement are usually negative.
5. Clinical course is aggressive (Figs 8-8A to G).

### *Acute Leukemias of Ambiguous Lineage*

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1. Leukemias with no clear evidence of single lineage differentiation.
2. Frequently associated with 11q23 or t(9;22) translocation.

Please refer to Table 13-1 regarding WHO requirement for assigning more than one lineage in a single blast population.



**Fig. 8-8A: Blastic plasmacytoid dendritic neoplasm.** Leukemic cells involving skin (Skin biopsy).



**Fig. 8-8B**

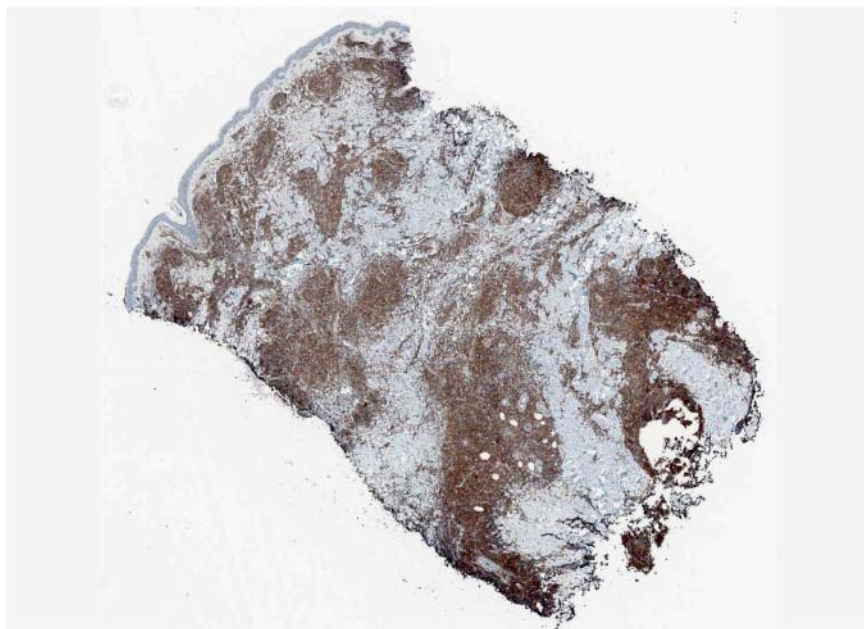
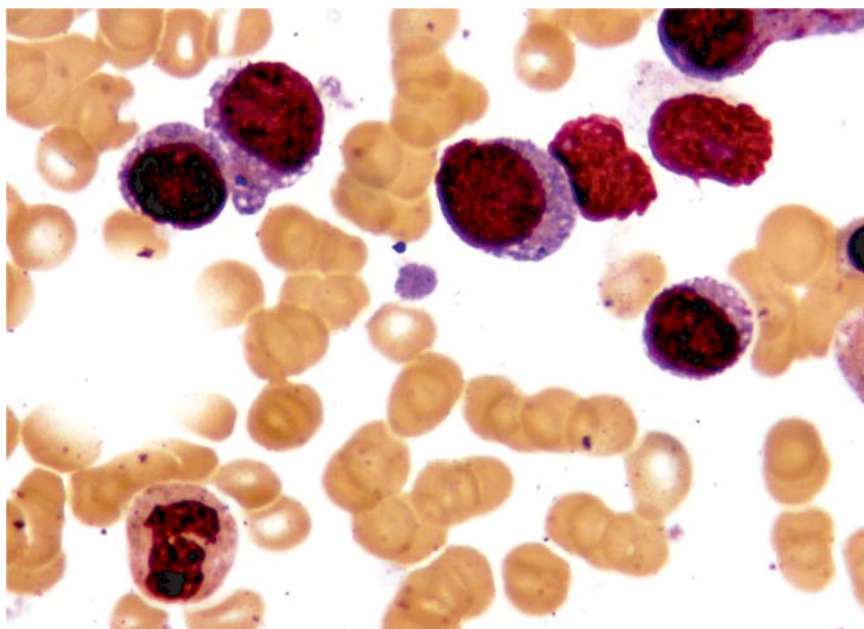


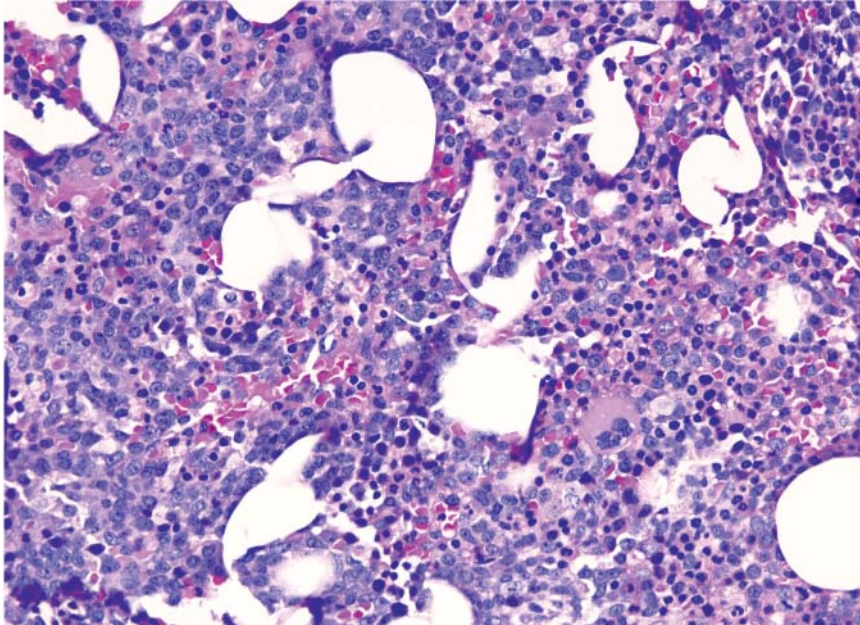
Fig. 8-8C

**Figs 8-8B and C: Blastic plasmacytoid dendritic neoplasm.** These neoplastic cells are CD4 (B) and CD56 (C) positive (Skin biopsy).

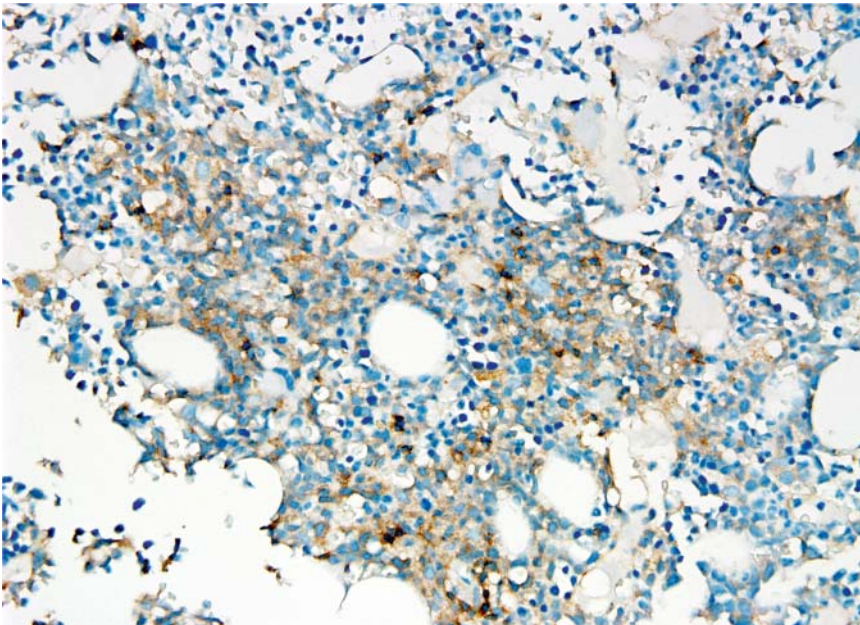


**Fig. 8-8D: Blastic plasmacytoid dendritic neoplasm.** Bone marrow aspirate showing neoplastic cells with eccentrically-located nuclei, finely-dispersed chromatin, abundant cytoplasm, and microvacuoles.





**Fig. 8-8E: Blastic plasmacytoid dendritic neoplasm.** Bone marrow section showing clusters of large atypical cells (left side).



**Fig. 8-8F**

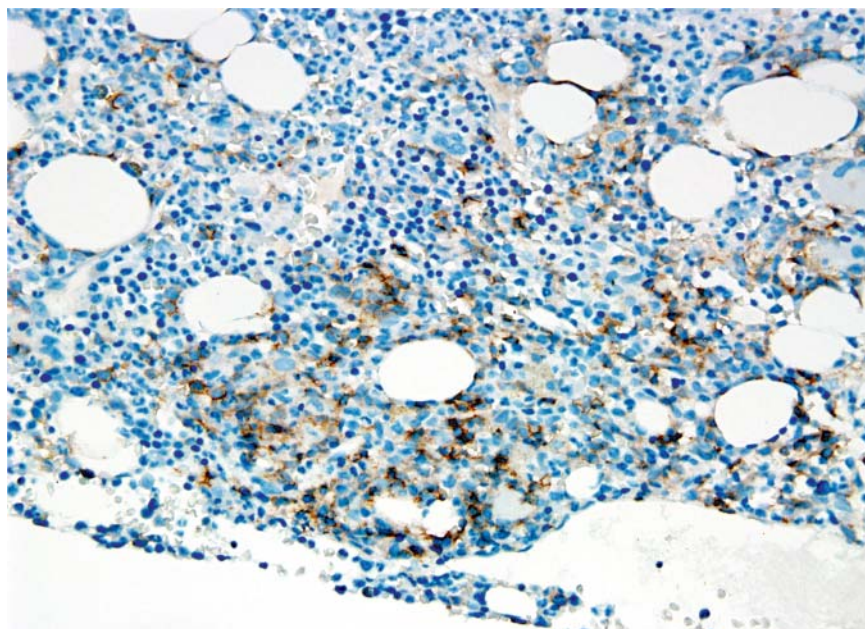


Fig. 8-8G

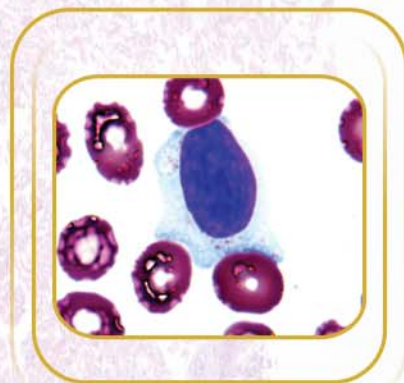
**Figs 8-8F and G: Blastic plasmacytoid dendritic neoplasm.** These neoplasm cells are CD4 (F) and CD56 (G) positive (Bone marrow section).



CHAPTER

9

# Acute Lymphoblastic Leukemia/Lymphoma, Mature T- and B-cell Leukemias



## *Acute Lymphoblastic Leukemia/Lymphoma (ALL)*

Clinical features include lethargy, fever, infection, bleeding, joint pain (typical early complaint from children), organomegaly, and cytopenia.

Seventy-five percent of ALL patients are under 6 years of age, and most patients are between 2-10 years old. Eighty-five percent of cases are B-ALL, and the rest are T-ALL.

## **WHO Classification of Precursor Lymphoid Neoplasms**

1. B acute lymphoblastic leukemia/lymphoma. Not otherwise specified.
2. B acute lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities.

Cytogenetic subtypes:

1. t(9;22)(q34;q11) BCR/ABL
2. t(v;11)(v;q11) MLL rearranged
3. t(1;19)(q23;p13) E2A-PBX (TCF3-PBX1)
4. t(5;14)(q31;q32) IL3-IGH
5. t(12;21)(p13;q22) TEL/AML-1
6. Hyperploidy (>50 chromosomes)
7. Hypoploidy (<46 chromosomes)
3. T acute lymphoblastic leukemia/lymphoma.

**TABLE  
9-1**

**Comparison of immunophenotype, cytochemistry and gene rearrangements of B- and T-ALL**

	B-ALL	T-ALL
MPO	—	—
NSE	—/+ (focal)	—/+ (focal)
PAS	+	+
Acid phosphatase	+	+
TdT	+	+
T associated antigens	—	+
B associated antigens	+	—
clg	—/+ (20% has $\mu$ chain)	—
slg	—	—
B IgH rearrangement	Near 100%	20%
T receptor rearrangement	Up to 70%	Near 100%

Note: B- or T-cell gene rearrangements cannot be used as a lineage assignment of ALL.

Please refer to Table 13-1 for lineage assignment.

**B-ALL**

1. Expression of various combinations of B-lymphocyte antigens CD19, CD22, CD79a, CD24, and CD10 (CD10 is usually negative in infants and negative in 25% of adult B-ALL cases).
2. CD20 is highly specific for B-ALL, but is usually absent or dim in many cases.
3. Surface immunoglobulin (sIg) is absent.
4. Cytoplasmic immunoglobulin m chain (cIg) is present in about 20% of B-ALL cases.
5. No expression of light chain.
6. Other non-specific antigens: TdT+ (90%), CD34+ (75%), HLA-DR+ (98%), CD38+ (98%), and CD45+ (70%).

**TABLE 9-2** Cytogenetic translocations and associated molecular abnormalities of ALL

Translocation	Molecular abnormalities	Children	Adult
<b>t(9;22)(q34;q11)</b> t(4;11)(q21;q23) t(1;19)(q23;p13)	<b>BCR-ABL fusion</b> MLL-AF4 fusion E2A(TCF3)-PBX1 fusion	2-4% of cases	25-30% of cases
<b>t(12;21)(p13;q22)</b> t(11;14)(p13;q11) t(5;14)(q13;q32) t(17;19)(q21-22;p13)	<b>TEL-AML1 fusion</b> TCRd-RBTN2 fusion IL-3/IgH E2A/HLF	20-27% of cases	3-4% of cases

**TABLE 9-3** Cytogenetics and associated clinical prognosis of ALL

Prognosis	Cytogenetic findings
Favorable	Hyperploidy (>50 chromosomes) t(12;21)
Intermediate	Hyperploidy (47-50 chromosomes) Normal (diploidy) del(6q) t(1;19)
Unfavorable	Hypodiploidy-near haploidy Near tetraploidy del 17 t(9;22) t(11q23) such as t(4;11) 9p abnormalities t(17;19) t(5;14)



7. IgH gene rearrangement is positive. However, up to 70% of cases also show a TCR gene rearrangement.
8. B-ALL has a better prognosis in children, but it is less favorable in adults.
9. The prognosis of B-ALL depends on cytogenetics, age, stage, and LDH level.

### **B-lymphoblastic Leukemia/Lymphoma with Recurrent Genetic Abnormalities**

#### ***t(9;22)(q34;q11.2)***

1. t(9;22) translocation is associated with older patients, high leukocyte counts, organomegaly, and CNS involvement.
2. t(9;22) translocation presents more commonly in adults.
3. t(9;22) translocation is associated with the worst prognosis among patients with ALL.

**The difference of t(9;22)(q34;q11.2) BCR-ABL fusion in CML and ALL:**

1. CML: translocation results in a **210-KD** protein (Major breakpoint).
2. ALL: translocation results in a **190-KD** protein (Minor breakpoint).

#### ***t(v;11q23) MLL Rearranged***

1. ALL with MLL rearrangements is the most common leukemia in infants <1 year of age.
2. t(4;11) is the most common t(v;11)(v;q11) MLL rearranged ALL. It is associated with high leukocyte counts, splenomegaly, and usually has a CD10 negative precursor B-ALL phenotype.
3. MLL rearrangements are associated with high-risk clinical features and poor prognosis. MLL rearrangements are also present in *de novo* AML, topoisomerase II inhibitor drug-related secondary AML, and biphenotypic, and bilinear leukemias.

#### ***t(1;19)(q23;p13.3)***

1. t(1;19) is found in 5-6% ALL cases and 25% of cIg+ ALL cases.
2. Flow cytometry analysis is characterized by a homogeneous expression of CD19, CD10, CD9, cytoplasmic, absent CD34, and absent or under-expression of CD20. It was once associated with a poor prognosis, but recent therapies have improved the outcome.

***t(12;21)(p13;q22)***

1. *t(12;21)* is found in 20-27% childhood B-ALL cases and 3-4% adult B-ALL cases.
2. Flow cytometry analysis is characterized by expression of CD19, CD10, CD34 (most cases) positive, and frequent expression of myeloid antigens (CD13, CD33). Lack of expression of CD9 and CD20.
3. Favorable prognosis

***t(5;14)(q31;q32)***

1. Rare, less than 1% of ALL cases.
2. Commonly associated with a striking eosinophilia in the peripheral blood.
3. Flow cytometry analysis is characterized by expression of CD19 and CD10.
4. Prognosis is unfavorable.

**T Acute Lymphoblastic Leukemia/Lymphoma**

T-ALL comprises about 15% childhood ALL cases and 25% adult ALL cases. In many treatment protocols a bone marrow blast count of >25% has been used to define leukemia; however, there is no agreed-upon lower limit for the percentage of blasts required to diagnose leukemia.

1. Immunophenotype: Tumor cells express various combinations of T-lymphocyte antigens (CD2, CD3, CD4, CD5, CD7, and CD8) and other non-specific antigens including TdT (>90%), CD34 (less common than B-ALL), and CD10 (approximately 20%).

CD3 surface expression is negative in 2/3 cases. Most cases have cytoplasmic CD3. CD3 is considered lineage specific.

CD1a and co-expression of CD4/CD8 are present in 1/3 of the cases.

2. Cytogenetic and molecular study: The most common translocations associated with T-ALL are *t(11;14)* and *t(10;14)*. 14q11 is the location of TCR  $\alpha$ - and  $\delta$ -gene. Fifty percent of the T-ALL chromosome abnormalities are related to this breakpoint. Other translocations involve the TCR  $\beta$ -gene at 7q35 and the TCR  $\gamma$ -gene at 7p14-15.

TCR gene rearrangement is positive. However, 20% of cases also show an IgH gene rearrangement.

3. Prognosis: T-ALL in childhood is generally considered a higher risk disease than B-ALL. The prognosis of T-ALL depends on cytogenetics, age, stage, and LDH level.

## Differential Diagnosis of ALL

### *Hematogones and B-ALL*

Hematogones are normal B-cell precursors and are found in small numbers in the bone marrow of infants and young children.

Increased numbers of hematogones may be seen in healthy individuals, in association with various diseases or in regenerative bone marrows (after chemotherapy or transplant).

**TABLE  
9-4**

**Phenotype comparison of hematogones and B-ALL**

	Hematogones	B-ALL
CD10	+	+/-
CD34/TdT	-/+	+
Clusters of >3 CD34/TdT + cells	no	yes
Flow cytometry	maturation spectrum	no maturation spectrum

### *Reactive Lymphocytosis*

1. Commonly seen in viral infections. Flow cytometry and serologic studies for EBV and CMV are required to rule out malignancy.
2. **Pertussis** can present with marked lymphocytosis; the lymphocytes are usually small and mature appearing without visible nucleoli.

### *Aplastic Anemia*

Bone marrow biopsy will distinguish aplastic anemia from ALL with pancytopenia.

### *Acute Myeloid Leukemia*

Flow cytometry, morphology and cytochemistry study will help to distinguish from ALL.

### *Burkitt Leukemia/Lymphoma*

Burkitt leukemia cells have a vacuolized appearance in the bone marrow aspirate smear in most of the cases.

**TABLE  
9-5****Comparison of Burkitt lymphoma/leukemia and B-ALL**

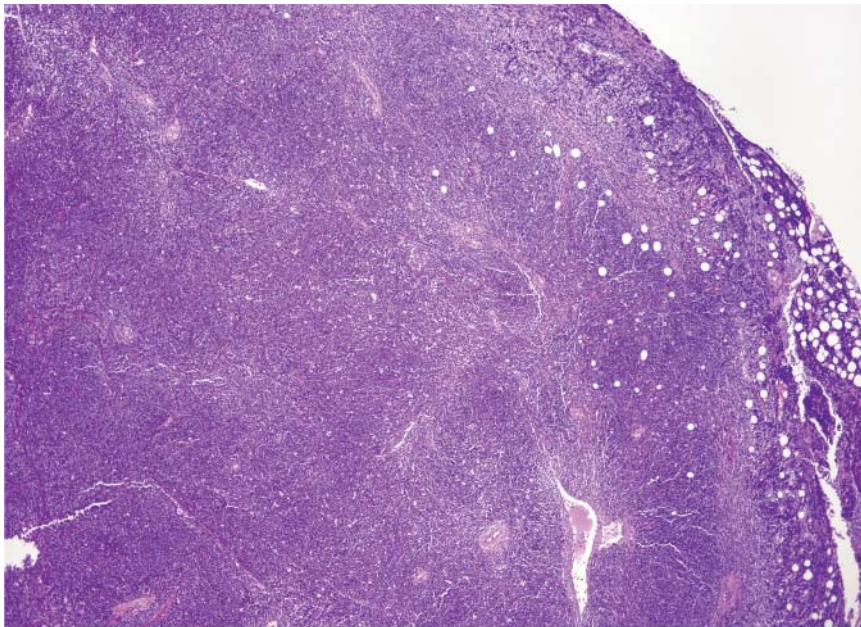
	Burkitt lymphoma	B-ALL
Pan B and CD10	Positive	Positive
slg	Monoclonal	No expression
TdT	Negative	Usually positive
Translocations	t(8;14) t(2;8) t(8;22)	Many

***Blastic Mantle Cell Lymphoma***

Flow cytometry, immunohistochemical stains and FISH will help to distinguish from ALL.

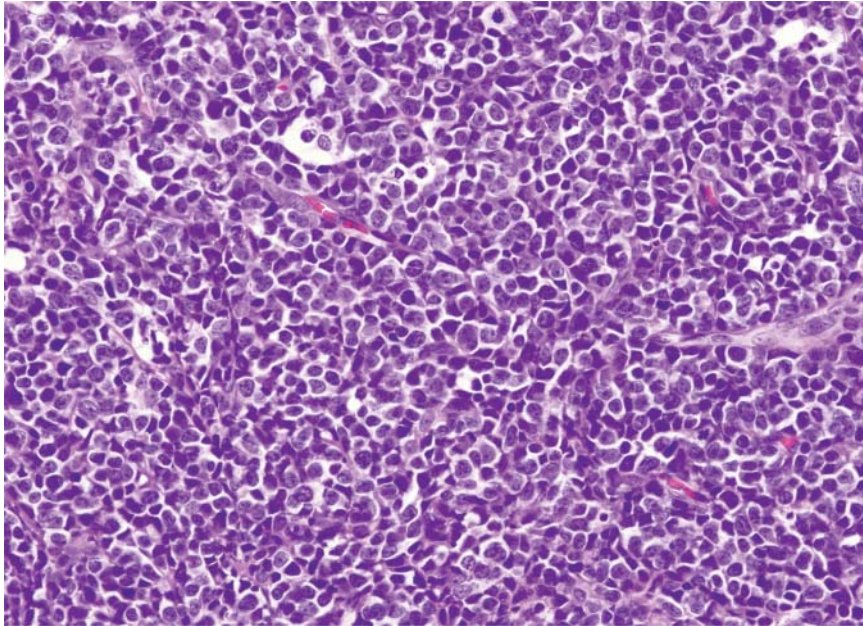
***Metastatic Small Cell Tumor***

Flow cytometry and/or immunohistochemical studies will help to distinguish from ALL (Figs 9-1A to D).

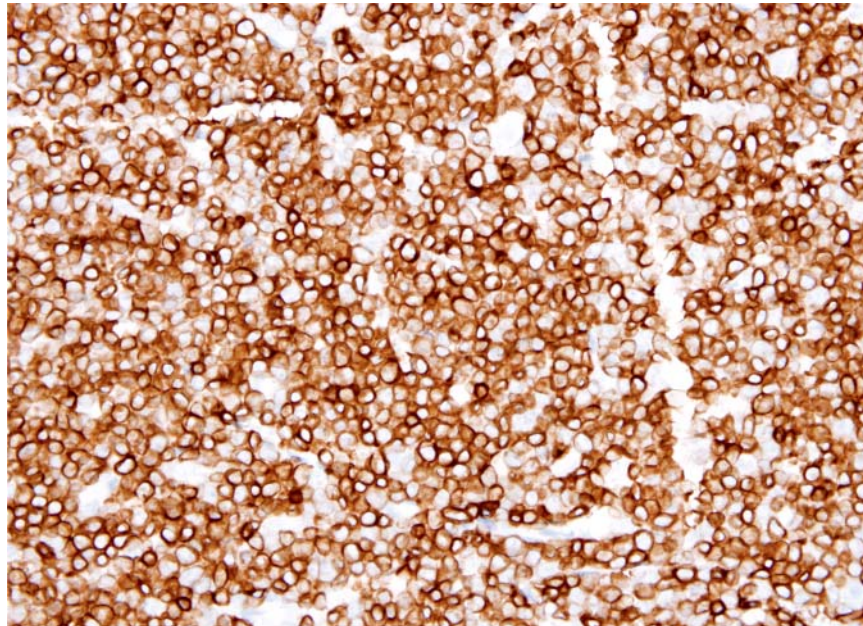


**Fig. 9-1A: T-cell acute lymphoblastic lymphoma.** The lymph node is totally effaced with infiltration into adjacent adipose tissue (Lymph node biopsy).



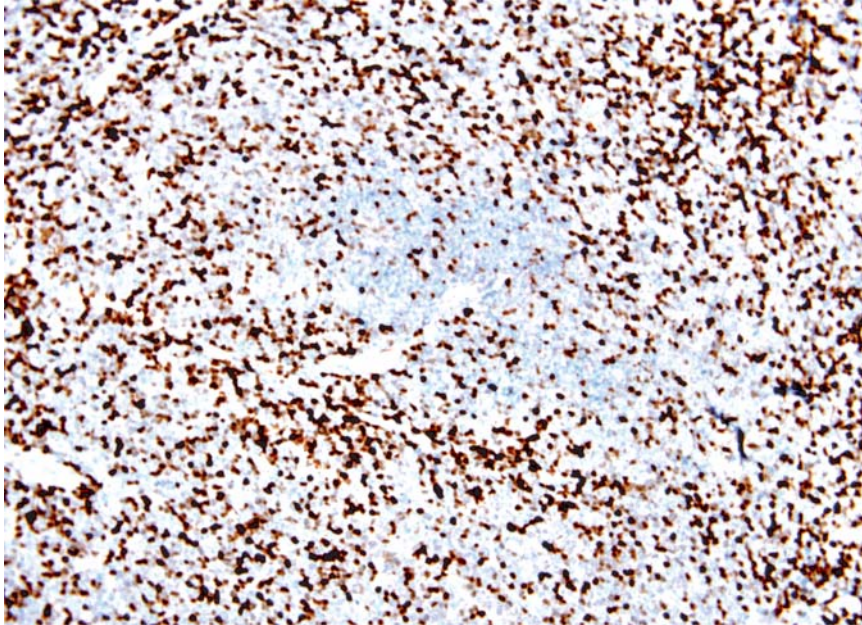


**Fig. 9-1B: T-cell acute lymphoblastic lymphoma.** At higher magnification, these cells range from small to medium size, with a very high nuclear to cytoplasmic ratio, condensed chromatin, and inconspicuous nucleoli (Lymph node biopsy).



**Fig. 9-1C: T-cell acute lymphoblastic lymphoma.** Immunohistochemical stain for CD3 (cytoplasmic stain) is positive (Lymph node biopsy).





**Fig. 9-1D: T-cell acute lymphoblastic lymphoma.** Immunohistochemical study for TdT (nuclear stain) is positive (Lymph node biopsy).

### *Mature B-cell Leukemia*

Classification of mature B-cell chronic lymphoid leukemias

1. Chronic lymphocytic leukemia (CLL).
2. B-cell prolymphocytic leukemia (PLL).
3. Hairy cell leukemia (HCL).

**TABLE  
9-6**

Comparison of typical flow cytometry study of mature B-cell leukemia/lymphoma

	CLL	PLL	HCL	SMZL	FL	MCL
Sig	+(dim)	+	+	+	+	+
CD5	+	—	—	—	—	+
CD10	—	—	—	—	+	—
CD19	+	+	+	+	+	+
CD20	+(dim)	+	+	+	+	+
CD23	+	+/-	—	—	—	—
FMC7	—	+	+	+	+	+
CD11c	+/-	—	+	+/-	—	—
CD25	—	—	+	—	—	—
CD103	—	—	+	—	—	—

## Chronic Lymphocytic Leukemia (CLL)/Small Lymphocytic Lymphoma (SLL)

CLL/SLL is the most common adult leukemia/lymphoma in western countries. It occurs in middle aged and elderly individuals, and the incidence increases with age. Male to female ratio is 2:1. Many patients are asymptomatic when the disease is diagnosed. Symptomatic patients present with weakness, fatigue, weight loss, fever, night sweats and/or frequent infections. Lymphadenopathy and splenomegaly may be present but are less common.

Monoclonal B cell lymphocytosis represents a mild expansion of monoclonal B-cells in otherwise healthy individuals. Lymphocytosis is less than 5000/ $\mu$ l ( $5 \times 10^9$ /L); these monoclonal B-cells usually have the phenotype of CLL.

1. Peripheral blood: Lymphocytosis, absolute lymphocyte count is usually  $>5000/\mu$ l ( $5 \times 10^9$ /L). **Smudge cells are common.**
2. Bone marrow: Usually hypercellular with a variable increase in lymphocytes.
3. Lymph node: Effacement of normal architecture by small lymphocytes. A pale nodular pattern of proliferation centers (pseudofollicles) is best observed when you dim the microscope's light source.
4. Flow cytometry: Leukemic cells are CD5+, CD19+, CD20+ (usually dim), CD23+, and FMC7- with a light chain restriction. IgM and IgD heavy chain are usually present but dim. IgG and IgA expression is much less common. Expression of CD38 and ZAP-70, and an un-mutated IgVH are associated with a poor prognosis.
5. Transformation of CLL (**Richter syndrome**): 2-8% of CLL cases progress to diffuse large B-cell lymphoma.
6.  $<1\%$  of CLL patients develop classical Hodgkin lymphoma (composite lymphoma).
7. Cytogenetic and molecular studies (Table 9-8).
8. Prognosis depends on expression of CD38 and ZAP-70, stage, status of IgVH mutation, and cytogenetic abnormalities (Figs 9-2A to F).

## B-cell Prolymphocytic Leukemia (B-PLL)

B-PLL is a rare mature B-cell leukemia. It comprises only 1% lymphocytic leukemia cases with a males to females ratio of 1:1. B-PLL patients are predominantly elderly individuals with a median age of 65-69 years old. **Splenomegaly is prominent in most patients, but lymphadenopathy is minimal or absent.**

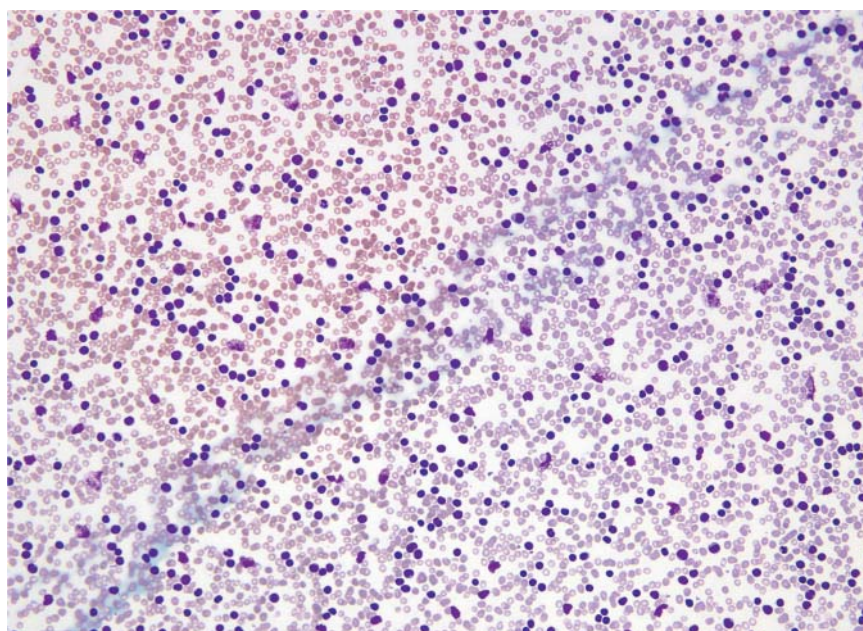
1. Peripheral blood: High WBC count ( $>100 \times 10^9$ /L) and marked lymphocytosis. **Prolymphocytes must exceed 55% of the total**

**TABLE  
9-7****Rai clinical staging of CLL/SLL**

Stage	Findings
0	Lymphocytosis in the blood and bone marrow only.
I	Lymphocytosis plus enlarged lymph nodes.
II	Lymphocytosis plus enlarged liver and/or spleen; lymphadenopathy may be present.
III	Lymphocytosis plus anemia (Hgb < 11 g/dl); lymph nodes, liver, and/or spleen may be enlarged.
IV	Lymphocytosis and thrombocytopenia (platelet < 100 K/ $\mu$ l), anemia and organomegaly may be present.

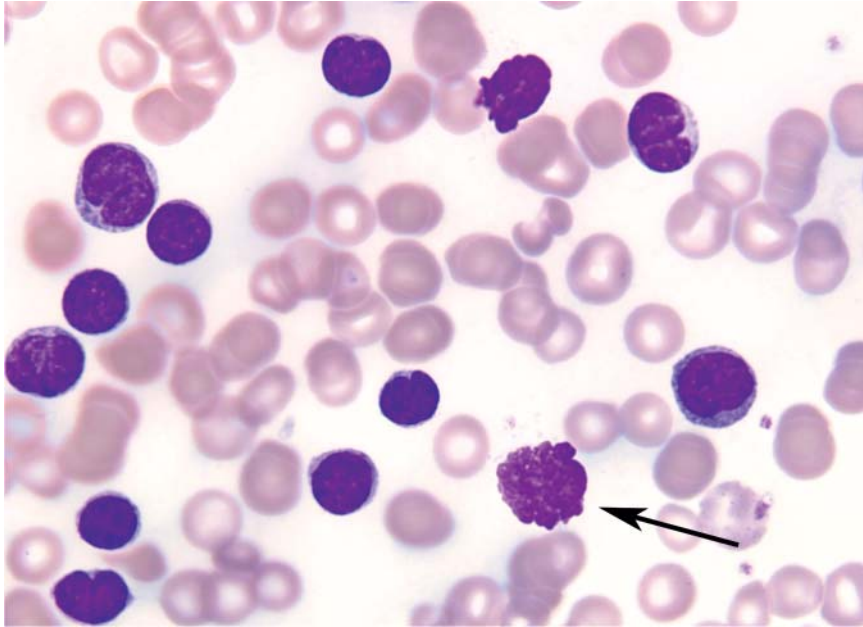
**TABLE  
9-8****Prognosis and cytogenetic abnormalities in CLL/SLL**

Favorable	<b>Deletion 13q14</b> (50% of the cases)
Unfavorable	<b>Trisomy 12</b> (20% of the cases) Deletion of 6q21-q23, 11q22-q23, 17p13 (11q and 17p are associated with more advanced disease and short survival times)

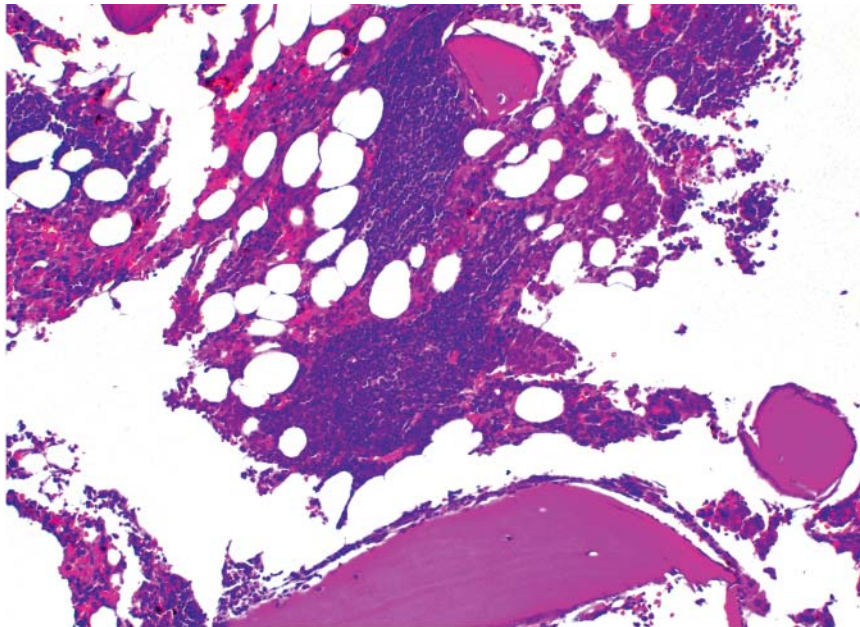


**Fig. 9-2A: Chronic lymphocytic leukemia (CLL).** Marked increase in small mature lymphocytes (Peripheral blood smear).

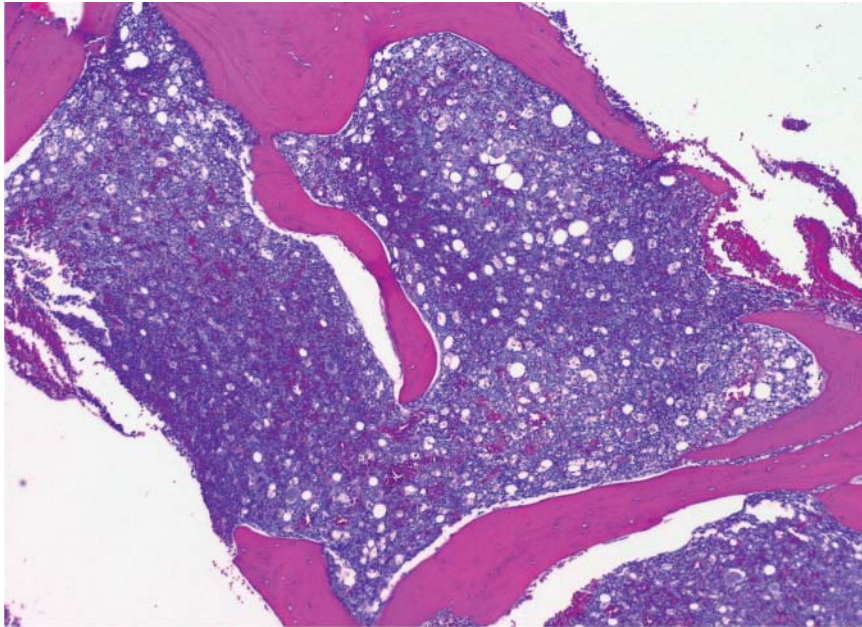




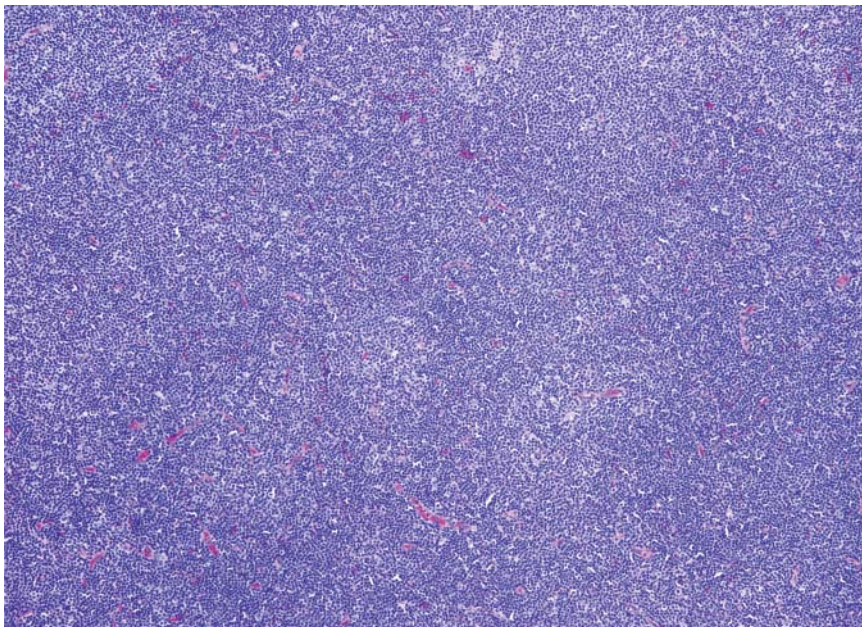
**Fig. 9-2B: Chronic lymphocytic leukemia (CLL).** Smudge cells (lymphocyte nuclear remnant, arrow) are characteristic of CLL. Smudge cells are artifact caused by shear forces during the preparation of the blood smear (Peripheral blood smear).



**Fig. 9-2C: Chronic lymphocytic leukemia (CLL).** CLL involvement of the bone marrow in a nodular pattern (Bone marrow section).

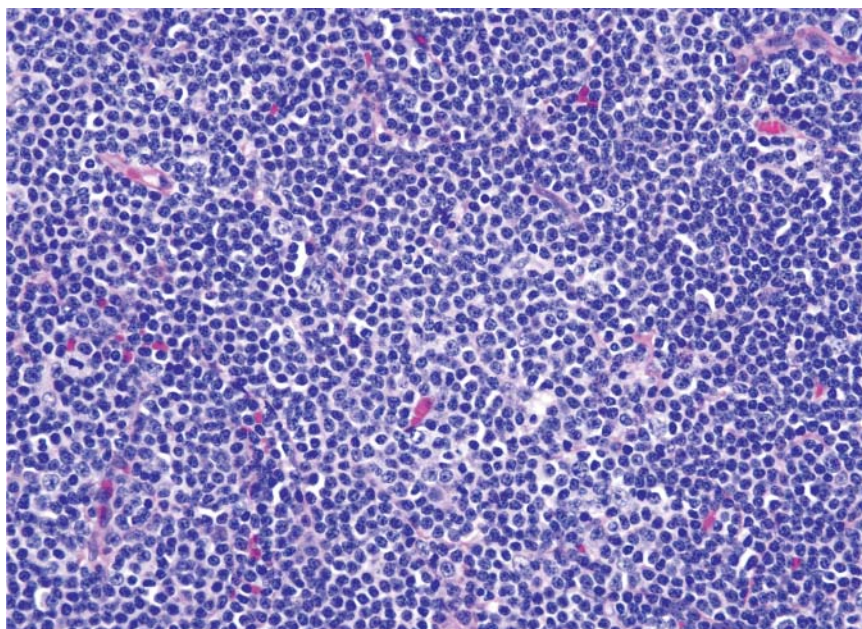


**Fig. 9-2D: Chronic lymphocytic leukemia (CLL).** CLL involvement of the bone marrow in a diffuse pattern (Bone marrow section).



**Fig. 9-2E: Small lymphocytic lymphoma (SLL).** The normal lymph node architecture is total effaced, a nodular pattern of pale proliferative centers (pseudo-follicles) are present in the center of the image (Lymph node biopsy).





**Fig. 9-2F: Small lymphocytic lymphoma (SLL).** Neoplastic B-cells are typical, small round lymphocytes with admixed polymorphocytes and paraimmunoblasts (Lymph node biopsy).

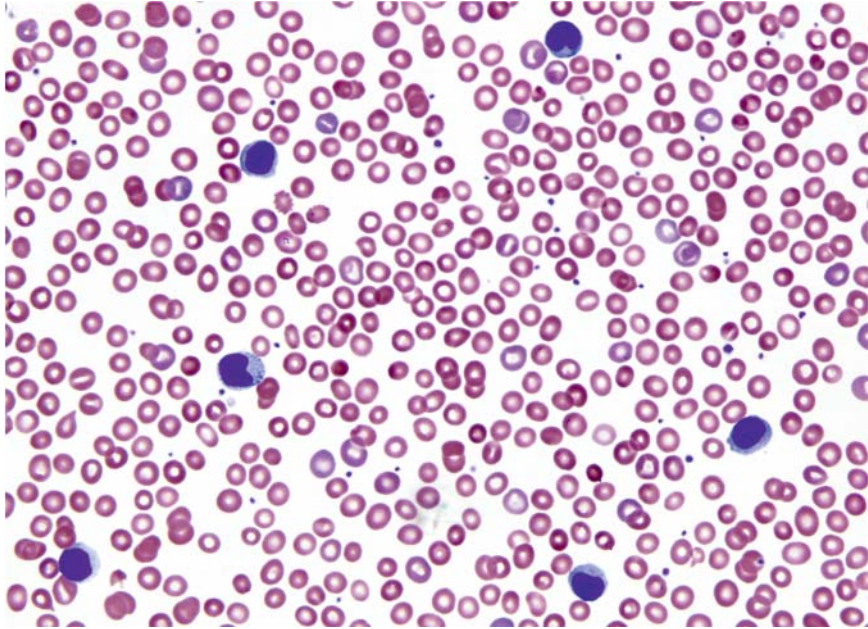
**lymphocytes to make the diagnosis.** Prolymphocytes are larger than CLL cells, the nucleoli are usually more prominent and some have folded nuclei.

2. Bone marrow: Usually hypercellular with a variable increase in the number of lymphocytes.
3. Flow cytometry: Leukemic cells are CD19+, CD20+ with light chain restriction. Unlike CLL/SLL, B-cell prolymphocytic leukemia surface immunoglobulin expression is bright and FMC7 is positive.
4. Cytogenetic and molecular studies: A complex karyotype is common; Del(17p) presents in 50% of the cases and associated with a TP53 mutation, 27% of cases show deletion of 13q14 by FISH probe and trisomy 12 is rare.

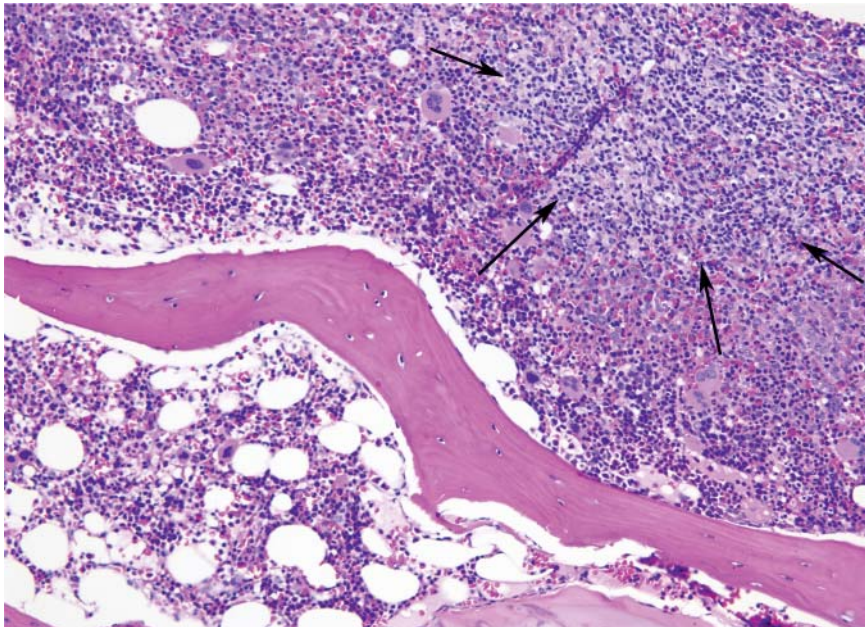
If t(11;14) translocation is present, the diagnosis should be mantle cell lymphoma, leukemic phase, not B-PLL (Figs 9-3A and B).

## Hairy Cell Leukemia

Hairy cell leukemia (HCL) is characterized by pancytopenia, splenomegaly, and minimal lymphadenopathy. The mean age is 50 years, and there is a male predominance. In rare cases, HCL may present as aplastic anemia. The



**Fig. 9-3A: Acute prolymphocytic leukemia.** The leukemic cells are larger than those seen in chronic lymphocytic leukemia, and some of them show large nucleoli and nuclear folds (Peripheral blood smear).



**Fig. 9-3B: Acute prolymphocytic leukemia.** Involvement of the bone marrow in a nodular pattern (arrows) (Bone marrow section).

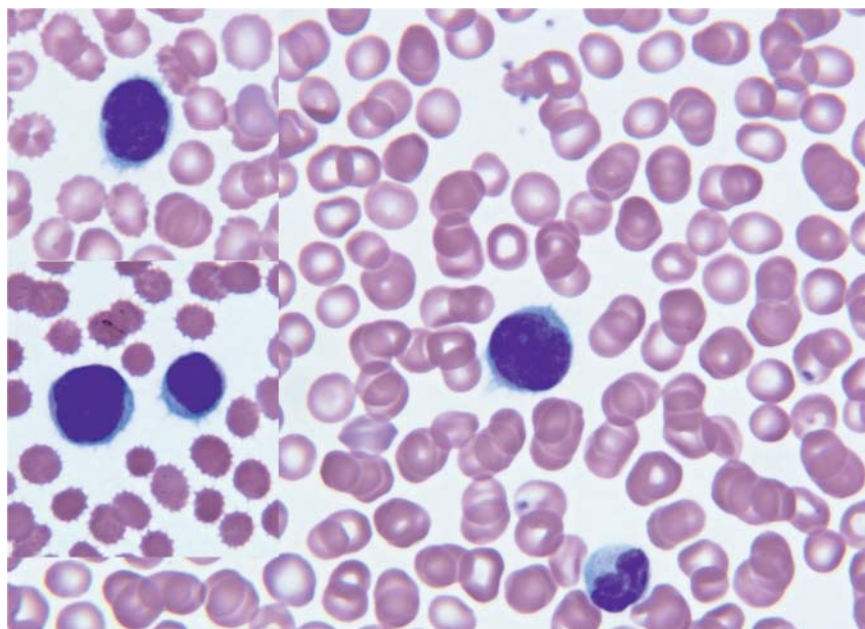


diagnosis of aplastic anemia in an adult should not be made without performing CD3, CD20, and DBA.44 stains on the bone marrow.

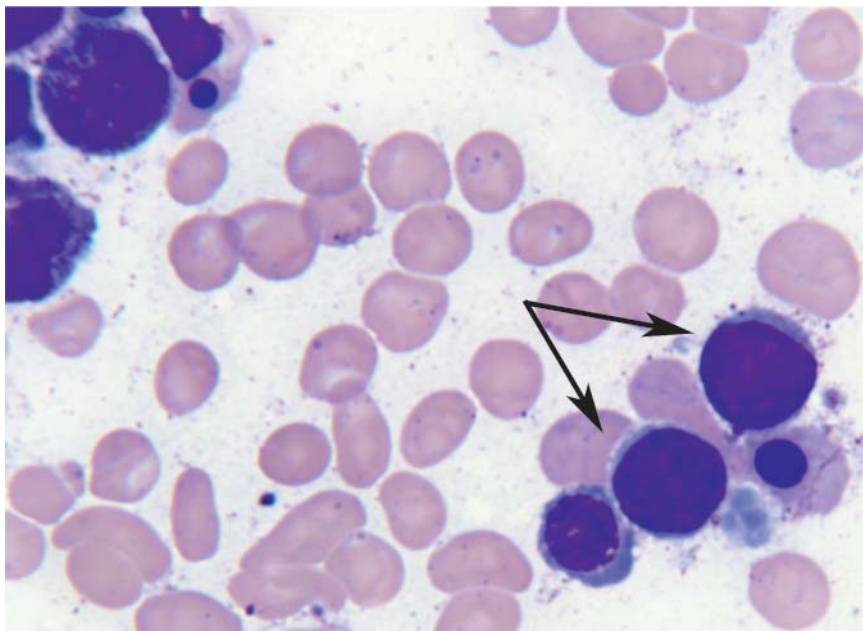
1. Spleen: Red pulp is infiltrated and expanded by leukemic cells.
2. Peripheral blood: Pancytopenia and “hairy” lymphocytes
3. Bone marrow: Infiltration of lymphocytes with abundant cytoplasm and prominent cell borders (“fried egg” appearance). Always perform CD20 or CD79a stain to rule out possible bone marrow involvement if morphologic evidence is absent.
4. Flow cytometry and laboratory studies: Hairy cells are usually positive for CD19, CD20, **CD11c**, **CD25**, **CD103**, **DBA.44**, and **Annexin-A1**, FMC7, and bright surface immunoglobulin.

Hairy cells are positive for cytochemical **TRAP** stain. Among B-cell lymphomas, Annexin has been only detected in hairy cell leukemia.

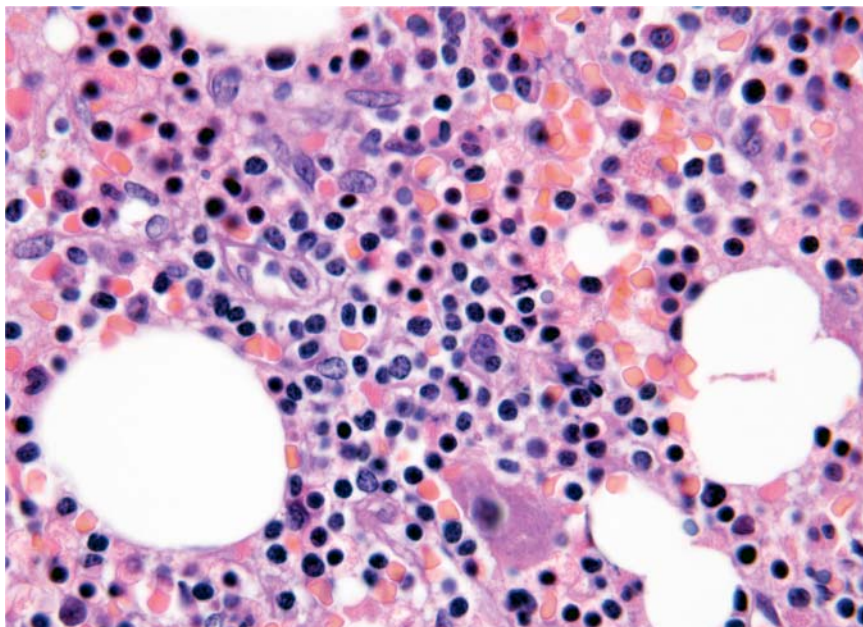
5. Cytogenetic and molecular studies: There are no specific cytogenetic abnormalities associated with HCL.
6. Hairy cell leukemia variant: Absence of CD25, Annexin-A1 and TRAP (Figs 9-4A to E).



**Fig. 9-4A: Hairy cell leukemia.** The leukemic cells show condensed nuclear chromatin with abundant cytoplasm and irregular cytoplasmic projections (Peripheral blood smear).

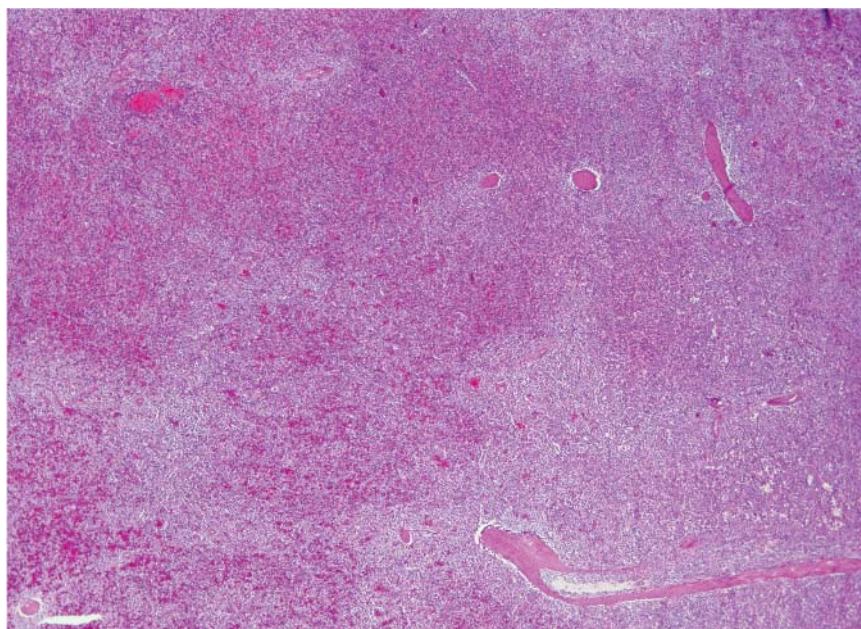


**Fig. 9-4B: Hairy cell leukemia.** The leukemic cells show irregular cell membranes and cytoplasmic projections (Bone marrow aspirate).

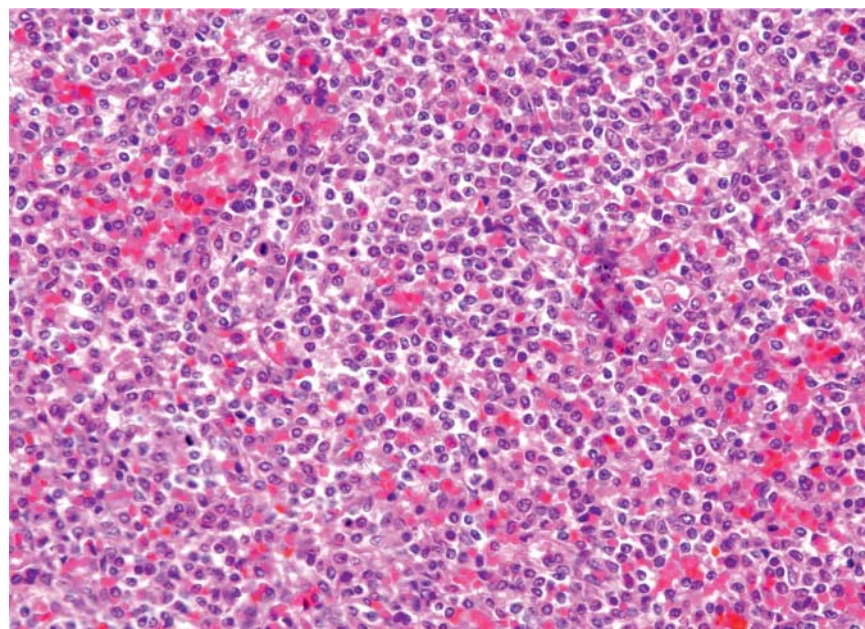


**Fig. 9-4C: Hairy cell leukemia.** The abundant cytoplasm around the nucleus gives the sheets of cells the appearance of "fried eggs" (Bone marrow section).





**Fig. 9-4D: Hairy cell leukemia.** Diffuse involvement of splenic red pulp, no white pulp is identified (Spleen).



**Fig. 9-4E: Hairy cell leukemia.** At higher magnification, the leukemic cells resemble those seen in bone marrow sections (Spleen).

## ***Mature T-cell Leukemia and Natural Killer Cell-Large Granular Lymphocyte Leukemia (NK-LGLL)***

### **Classification**

1. Classification of T-cell chronic lymphoid leukemias.
  - a. T-cell prolymphocytic leukemia (T-PLL)
  - b. T-cell large granular lymphocyte leukemia (T-LGLL)
  - c. Adult T-cell leukemia/lymphoma (ATLL)
  - d. Sézary syndrome (SS).
2. Natural killer cell-large granular lymphocyte leukemia (NK-LGLL).

**TABLE  
9-9**

**Comparison of typical flow cytometry study of T-cell and NK-cell leukemia**

	T-PLL	T-LGLL	NK-LGLL	ATLL	SS
CD2	+	+	+	+	+
CD3	+	+	—	+	+
CD7	+	+	—	+	+
CD4	+	—	—	+	+
CD8	+/-	+	—	—	—
CD7	+	+/-	—	—	—
CD25	+/-	—	—	+	—
CD56		—	-/+		
CD57		+	+/-		

### **T-cell Prolymphocytic Leukemia (T-PLL)**

1. Rare and aggressive
2. Present with hepatosplenomegaly and lymphadenopathy
3. Flow cytometry: Leukemic cells express pan T-cell markers (CD2, CD5, CD7), plus the following:
  - CD4+, CD8- (65%)
  - CD4+, CD8+ (20%)
  - CD4-, CD8+ (15%).
4. Cytogenetic and molecular studies: >80% abnormally involve 14q11, 14q32.1, inv(14)(q11;q32.1). 14q11 is the location of TCR  $\alpha$ - and  $\delta$ -gene.
  - TCR gene rearrangement is positive.

### T-cell Large Granular Lymphocytic Leukemia (T-LGLL)

T-LGLL is a rare leukemia. It is characterized by persistent (>6 months) mild to moderate lymphocytosis, **neutropenia** (80% of the patients), polyclonal hypergammaglobulinemia, mild to moderate splenomegaly, and **absent (or very rare) lymphadenopathy**.

1. Peripheral blood: Increased number of large granular lymphocytes ( $>2000/\mu\text{l}$  or  $2 \times 10^9/\text{L}$ ).
2. Bone marrow: Variable involvement, usually less than 50% of bone marrow cellularity. Leukemic cells are difficult to visualize by light microscope.
3. Flow cytometry: Mature post-thymic phenotype CD3+, most are CD8+, CD4- (rare CD4+ /CD8- or CD4+/CD8+), frequent loss of expression of CD5, CD7, and aberrant co-expression of CD16 and CD57.
4. Cytogenetic and molecular studies: TCR gene rearrangement is positive (Figs 9-5A and B).

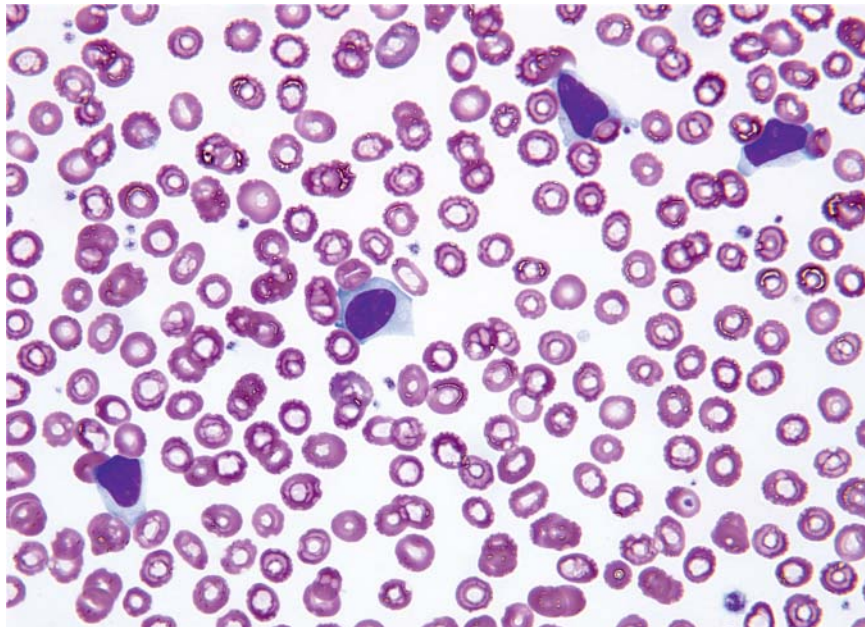
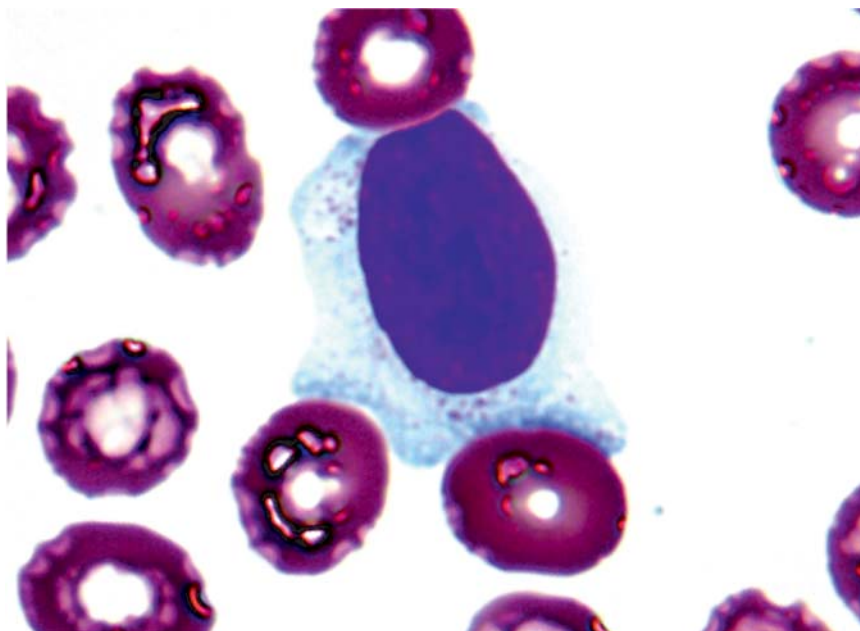


Fig. 9-5A: Large granular lymphocytic leukemia showing lymphocytosis (Peripheral blood smear).





**Fig. 9-5B: Large granular lymphocytic leukemia.** At higher magnification, the lymphocytes contain abundant cytoplasm and eosinophilic cytoplasmic granules (Peripheral blood smear).

### NK-Large Granular Lymphocytes Leukemia

NK-large granular lymphocytes leukemia (NK-LGLL) is listed as a provisional entity of chronic lymphoproliferative disorders of NK cells in WHO 2008 classification. It is an indolent disorder of NK lymphocytes with clinical and morphological features similar to T-LGLL.

1. Peripheral blood: Increased numbers of large granular lymphocytes ( $>2000/\mu\text{l}$  or  $2 \times 10^9/\text{L}$ ).
2. Flow cytometry: Leukemic cells are CD3-, CD2+, CD4-, CD8-, CD16+. CD56 expression is usually diminished or absent. CD57 expression is variable.
3. Cytogenetic and molecular studies: TCR gene rearrangement is negative (as expected for NK cells).

### Aggressive NK-cell Leukemia

Aggressive NK-cell leukemia is a neoplastic proliferation of NK-cells that is usually associated with EBV infection and an aggressive clinical course. Patients are young to middle aged adults. There is an increased prevalence in the Asian population.



**TABLE  
9-10****Comparison of T-LGLL and NK-LGLL**

	<b>T-LGLL</b>	<b>NK-LGLL</b>
CD2	+	+
CD3	+	–
CD4	–	–
CD5	–/+	–
CD8	+	–
CD7	–/+	–
CD16	+	+
CD56	–	–/+
CD57	+	– or weak
T-cell clonality	+	–

1. Clinical features: Cytopenia, increased LDH, hepatosplenomegaly, hemophagocytic syndrome, coagulopathy, and multiple organ failure.
2. Flow cytometry: Leukemic cells are CD2+, CD3ε+ (cytoplasmic CD3), CD16+, CD56+, surface CD3–, and CD57–, which is identical to extranodal NK/T-cell lymphoma, except the expression of CD16 is present in the majority of aggressive NK-cell leukemia cases (75%).
3. Cytogenetic and molecular studies: TCR gene rearrangement is negative

**TABLE  
9-11****Comparison of aggressive NK-cell leukemia and extranodal NK/T-cell lymphoma**

	<b>Aggressive NK-cell leukemia</b>	<b>Extranodal NK/T-cell lymphoma</b>
CD2+, CD3–, CD3ε+, CD56+, CD57– CD16	Yes 75% positive	Yes Usually negative
7p–, 17p–, 1q+	Frequent	Rare
6q–	Rare	Frequent

## Adult T-cell Leukemia/Lymphoma

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm caused by human T-cell leukemia virus type 1 (HTLV1). Most of the ATLL patients live or originate from HTLV-1 infection endemic areas. These areas include Asia (Southwestern Japan), the Caribbean basin, and Central Africa. Patients are usually exposed to the virus early in life. The virus can be transmitted through breast milk, blood, or blood products. Patients are usually

present with hypercalcemia, lytic bone lesion, cutaneous lesions, generalized lymphadenopathy, hepatosplenomegaly, and interstitial pulmonary infiltrates. The median age of patients is 55 years old.

The diagnosis of ATLL is made by clinical presentation, histologic identification of leukemic cells and detection of serum antibodies to HTLV-1.

1. Peripheral blood: Leukemic cells vary in size from small to large with marked irregular nuclei contours and deep nuclear indentations (flower cells). The N:C ratio is high without cytoplasmic granules. Leukemic cells also frequently infiltrate lymph nodes, skin, liver, and spleen.
2. Bone marrow: Involvement is patchy.
3. Flow cytometry: Leukemic cells have a phenotype of CD2+, CD3+, CD5+, CD7-, CD4+/CD8-. In rare cases, leukemic cells can be CD4-/CD8+ or CD4+/CD8+.

CD25 is strongly expressed in the majority of cases.

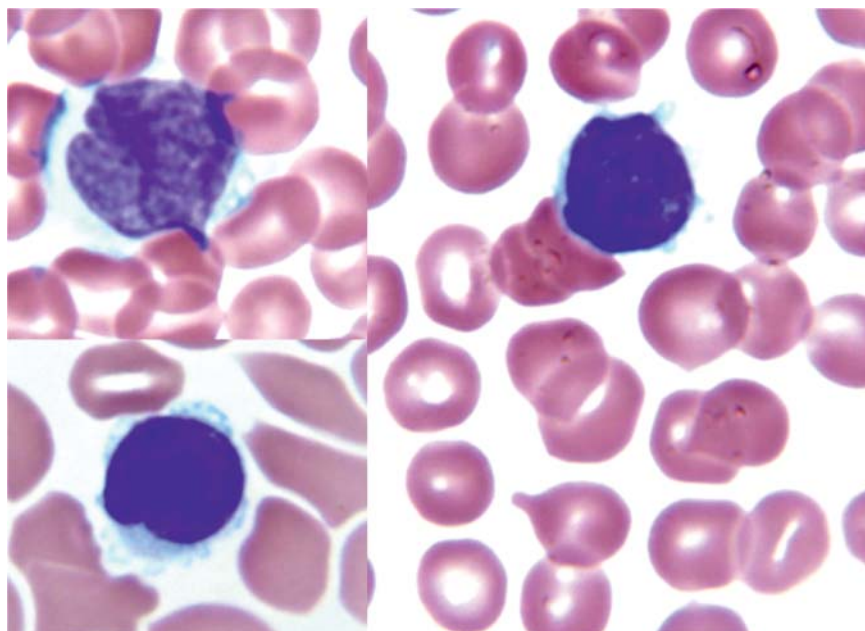
4. Cytogenetic and molecular studies: TCR gene rearrangement is positive.

## Sézary Syndrome

Sézary syndrome (SS) is a leukemic variant of cutaneous T-cell lymphoma and features a clinical triad of erythroderma, generalized lymphadenopathy and the presence of Sézary cells in the peripheral blood. Sézary syndrome may occur *de novo* or in association with mycosis fungoides.

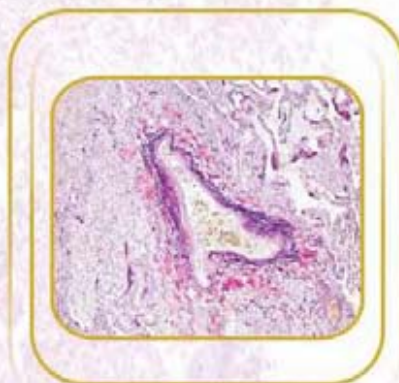
The diagnosis of Sézary syndrome is based on clinical triad of erythroderma, generalized lymphadenopathy and presence of clonal T-cell population (Sézary cells) in the peripheral blood plus:

1. An absolute Sézary cell count  $>1000/\mu\text{L}$  ( $1 \times 10^9/\text{L}$ ).
2. Immunophenotype abnormalities: An expanded CD4 population results in a CD4/CD8 ratio  $\geq 10$ , and/or loss of one or more pan T-cell markers.
  - a. Peripheral blood: Sézary cells show marked convoluted nuclei (cerebriform appearance).
  - b. Flow cytometry: Leukemic cells are CD2+, CD3+, CD5+, and characteristically CD7-, CD26-. Most cases are CD4+/CD8-. Rare cases are CD4-/CD8+ or CD4+/CD8+.
  - c. Cytogenetic and molecular studies: TCR gene rearrangement is positive (Fig. 9-6).



**Fig. 9-6: Sézary syndrome.** Sézary cells showing nuclear indentation, folds, and clefts (Peripheral blood smear).

# Non-Hodgkin Lymphomas, Plasma Cell Neoplasms and Histiocytic/Dendritic Cell Neoplasms





## *Non-Hodgkin Lymphomas*

Non-Hodgkin lymphomas are a heterogeneous group of malignancies. Their clinical presentation and course vary from indolent to aggressive. Indolent lymphomas are often disseminated at the time of diagnosis and frequently involve bone marrow.

Over the last decade, significant progress has been made in the molecular characterizations of lymphomas, but the classification of lymphomas is still evolving. The current WHO classification 2008 edition is listed in Appendix.

The prognosis of non-Hodgkin lymphomas depends on the type, the stage, the cytogenetic and molecular features (Table 10-1).

**TABLE  
10-1**

**Stages of lymphoma**

Stage	Findings
I	Disease limited to 1 anatomic region or 2 contiguous regions on the same side of the diaphragm
II	Disease in more than 2 anatomic regions or in non-contiguous regions on the same side of the diaphragm
III	Disease on both sides of the diaphragm, but limited to involvement of lymph nodes and spleen
IV	Disease of any lymph node region with involvement of liver, lung and bone marrow.

## **Mature B-cell Lymphomas with Small to Medium Sized Lymphocytes**

Lymphomas in this group are composed of small to medium sized lymphocytes. The most common lymphomas are chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), marginal zone lymphoma (MZL), follicular lymphoma (FL), mantle cell lymphoma (MCL), and lymphoplasmacytic lymphoma (LPL). The immunophenotypes of these lymphomas are listed in Table 10-2.

### ***Lymphoplasmacytic Lymphoma***

Lymphoplasmacytic lymphoma (LPL) is a rare indolent lymphoma that usually occurs in older patients and involves the lymph nodes, spleen and bone marrow. Patients present with weakness, fatigue and anemia. Most of the affected patients have a **monoclonal IgM** paraprotein; however, the presence

**TABLE  
10-2****Comparison of immunophenotype of small B-cell lymphomas**

	CLL/SLL	MZL	FL	MCL	LPL
Sig	+(dim)	+	+	+	+
CD5	+	—	—	+	—
CD10	—	—	+	—	—
CD20	+(dim)	+	+	+	+
CD23	+	—	—	—	—
FMC7	—	+	+	+	+
Cyclin D1	—	—	—	+	—

of a monoclonal IgM paraprotein is not required for diagnosis. If hyperviscosity syndromes are present, these findings are consistent with **Waldenström macroglobulinemia**. The diagnostic criteria of Waldenström macroglobulinemia is defined as lymphoplasmacytic lymphoma with bone marrow involvement and the presence of an IgM monoclonal gammopathy. Sometimes the distinction between lymphoplasmacytic lymphoma and marginal zone lymphoma is not always clear-cut; some cases may need to be diagnosed as small B-cell lymphoma with plasmacytic differentiation and a differential diagnosis provided.

1. Peripheral blood: Lymphocytosis may be present, but the WBC count is lower than that of CLL.
2. Bone marrow: Frequently involved by small lymphocytes admixed with plasma cells and plasmacytoid lymphocytes. Increased mast cells are often present.
3. Flow cytometry: Positive for pan B-cell markers (CD19, CD20), CD43+/-, CD5-, CD10-, and CD23-. Strong surface immunoglobulin usually IgM+, IgD-.
4. Cytogenetic and molecular studies: No specific chromosomal abnormalities are associated with lymphoplasmacytic lymphoma. Del 6q (40-60% of cases), 9p13 (related to PAX5 gene) and several others (including 14q32) may be present.

### ***Nodal Marginal Zone Lymphoma***

Nodal marginal zone lymphoma (NMZL) morphologically resembles extranodal or splenic type marginal zone lymphoma but without evidence of extranodal or splenic disease. Most of the patients are asymptomatic with localized or generalized lymphadenopathy.

1. Peripheral blood: Occasionally involved.

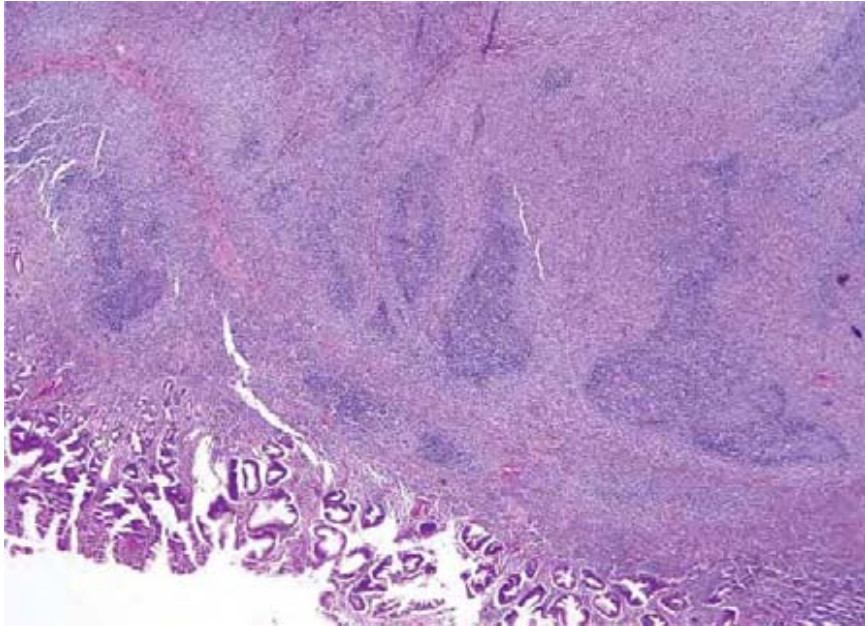
2. Bone marrow: Occasionally involved.
3. Lymph node: Lymphoma cells surround reactive follicles and expand into the interfollicular areas and/or the follicle. The lymphoma cells are composed of marginal zone B-cells, plasma cells and transformed B-cells.
4. Flow cytometry and immunohistochemistry: Lymphoma cells express pan B-cell markers (CD19, CD20), BCL2 (most of the cases), and CD43 (50% of the cases) with light chain restriction. CD5, CD10, CD23, BCL6, and Cyclin D1 are negative. IgD is positive in a minority of the cases.
5. Cytogenetic and molecular studies: Trisomy 3, 7 and 18 have been reported. The translocations of MALT lymphoma are *not* associated with nodal marginal zone lymphoma. IgH gene rearrangement is positive.

### ***Extranodal Marginal Zone B-cell Lymphoma of Mucosa-associated Lymphoid Tissue Type***

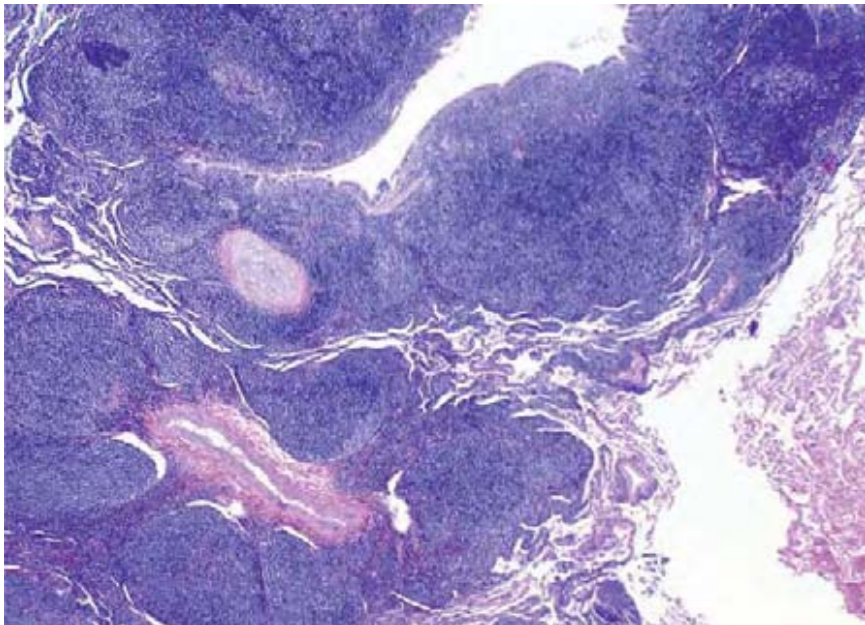
Mucosa-associated lymphoid tissue type (MALT) lymphoma comprises 7-8% of all B-cell lymphomas and up to 50% of the primary gastric lymphoma. The gastrointestinal tract is the most common site of MALT lymphoma.

Most of the cases occur in adults with a median age of 61 and a male to female ratio of 1:1.2. Patients often have a history of an autoimmune disease (i.e. Sjögren syndrome and Hashimoto thyroiditis), chronic inflammatory disorders or infection. Infections include *H. pylori* (stomach), *Borrelia burgdorferi* (skin) and *Chlamydia psittaci* (conjunctiva). Autoimmune or chronic inflammatory disorders result in an accumulation of extranodal lymphoid tissue that form precursor lesions. The diagnosis is based on morphology, flow cytometry, cytogenetics, and molecular studies.

1. Peripheral blood and bone marrow: Involvement is rare.
2. Mucosa-associated lymphoid tissue: The lymphoma is composed of morphologically heterogeneous small lymphocytes including marginal zone cells, scattered immunoblasts, centroblast-like cells, and plasma cells.
3. Flow cytometry and immunohistochemistry: Lymphoma cells express IgM (less often IgA, IgG) and pan B-cell markers (CD19, CD20) with light chain restriction. CD43 may be positive. CD5, CD10, CD23, BCL6, and Cyclin D1 are negative.
4. Cytogenetic and molecular studies:  
**t(11;18)(q21;q21)** is commonly associated with pulmonary, gastric and intestinal involvement. However, this abnormality is extremely rare in splenic marginal zone lymphoma.

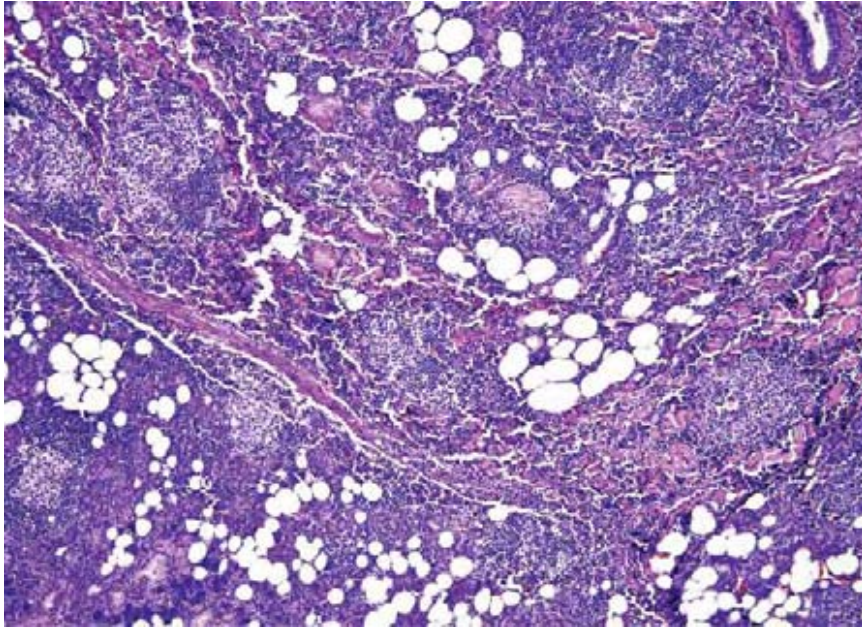


**Fig. 10-1A: Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue.** Diffuse small lymphocytic infiltration in the gastric mucosa, a site normally devoid of lymphocytes (Stomach).

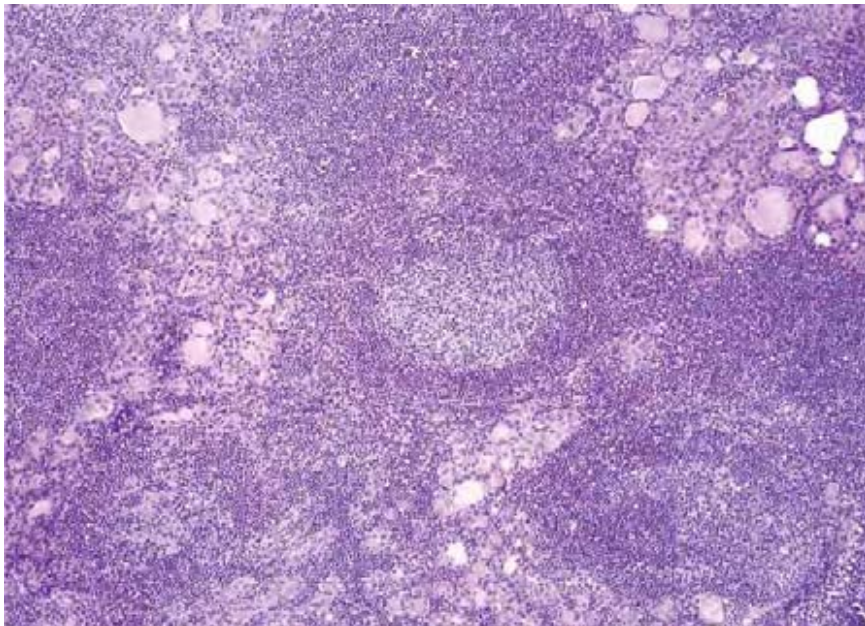


**Fig. 10-1B: Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue.** Small lymphocytes expanding the interstitium of the lung, forming nodules (Lung).





**Fig. 10-1C: Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue.** Lymphoepithelial lesions are characteristic of MALT lymphoma (Parotid gland from a patient with Sjögren syndrome).



**Fig. 10-1D: Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue.** Small lymphocytes diffusely infiltrate the thyroid gland (Thyroid from a patient with Hashimoto thyroiditis).

**t(1;14)(p22;q23)** involves BCL-10 gene. t(11;18) and nuclear stain of BCL-10 are associated with disseminated disease and antibiotic resistance.

**t(14;18)(q32;q21)** is commonly associated with ocular and salivary gland involvement.

**t(3;14)(p14.1;q32)** is commonly associated with thyroid, ocular and skin involvement.

**Trisomy 3, trisomy 18 and t(1;14)(p22;q32)** are also associated with MALT lymphoma (Figs 10-1A to D).

### ***Follicular Lymphoma***

Follicular lymphoma (FL) comprises of 20% of all lymphoma. The median age is in the 6th decade of life with a male to female ratio of 1:1.7. Follicular lymphoma is rare in individuals under 20 years old.

1. Peripheral blood: Occasional lymphocytosis with small-cleaved lymphocytes
2. Bone marrow: **Paratrabecular pattern** of lymphoid aggregates. The aspirate may be negative due to lymphocytes sticking to the trabecular bone.
3. Lymph node: Back-to-back follicular nodules replacing the normal nodal architecture.
4. Flow cytometry: Lymphoma cells are CD10, CD19, CD20, CD79a, BCL2, and BCL6 positive with light chain restriction. Unlike CLL/SLL, follicular lymphoma surface immunoglobulin expression is bright and FMC7 is positive. CD5, CD11c, CD23, CD25, and CD103 are negative.
5. Cytogenetic and molecular studies: Characteristic **t(14;18)(q32;q21)**. Other genetic abnormalities may also present:
  - +7
  - +18
  - 3q27-28 (up to 30% in grade 3B)
  - 17p (adverse prognosis)
  - 6q23-26 (adverse prognosis)

Some cases of diffuse large B-cell lymphoma that have transformed from follicular lymphoma may contain a cMYC translocation (“**double-hit**” lymphoma) or a cMYC and a 3q27/BCL6 translocation (“**triple-hit**” lymphoma), which is associated with a poor prognosis. In rare cases, follicular lymphoma may transform to histiocytic dendritic sarcoma.

The key feature to distinguish normal follicular hyperplasia from follicular lymphoma is to compare CD10 and MIB-1 immunohistochemical stains. Follicular hyperplasia has a dense sharply demarcated follicular

CD10 and MIB-1 staining pattern opposed to follicular lymphoma, which has a light CD10 and scattered MIB-1 follicular staining pattern.

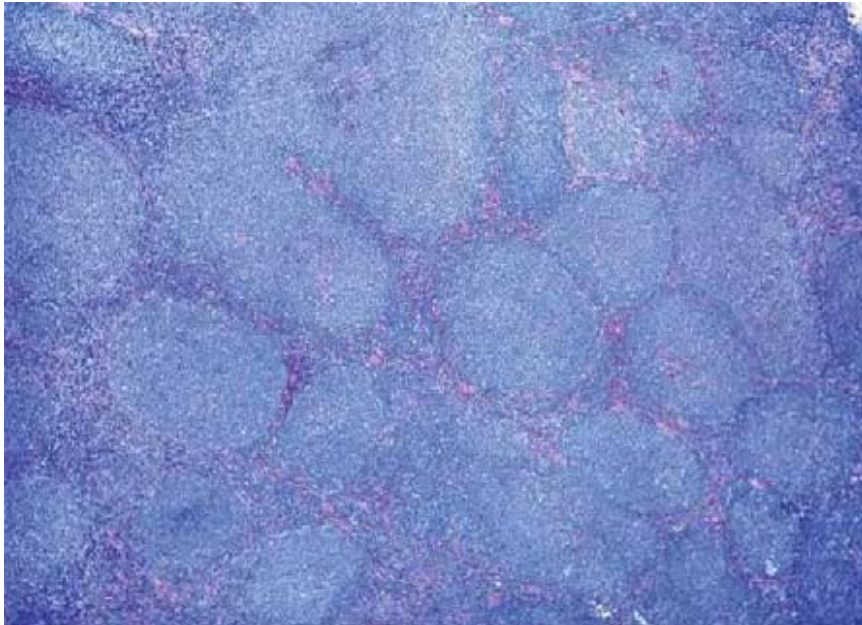
6. Grading of follicular lymphoma:

- **Low grade** (grade 1-2, same clinical outcome. Median survival is about 10 years and is unaffected by aggressive treatment. 20% of low grade FL transforms to DLBCL)  
 Grade 1: 0-5 centroblasts per HPF (40x objective)  
 Grade 2: 6-15 centroblasts per HPF (40x objective)
- **High grade** (median survival is 2-6 years, frequently progresses to DLBCL)  
 Grade 3  
 3A: >15 centroblasts per HPF (40x objective)  
 3B: solid sheets of centroblast (DLBCL).

7. Reporting of pattern:

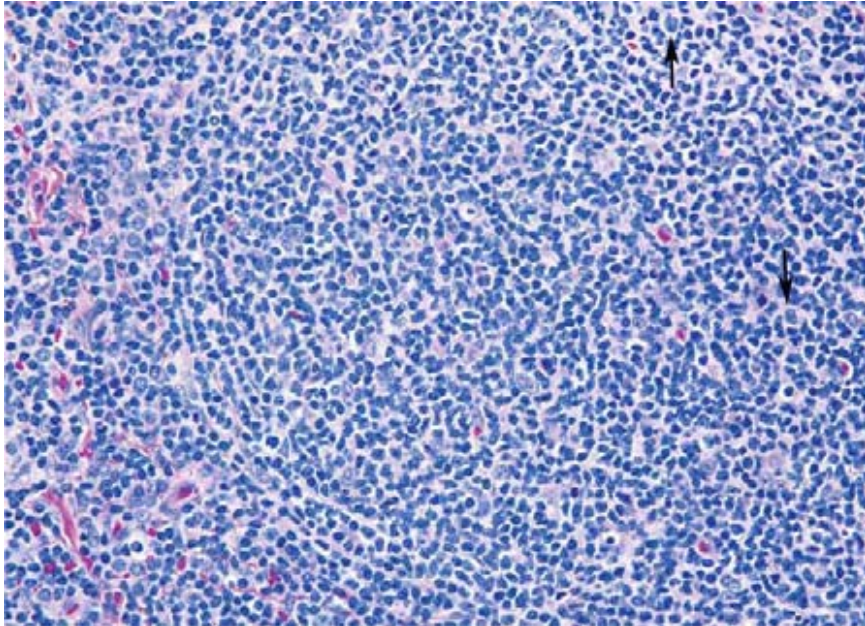
Follicular: >75% follicular pattern  
 Follicular and diffuse: 25-75% follicular pattern  
 Focal follicular: <25% follicular pattern  
 Diffuse: diffuse, no follicular pattern identified.

8. Follicular lymphoma variant: Diffuse follicular lymphoma is a variant of follicular lymphoma with an entirely diffuse growth pattern (Figs 10 -2A to H).

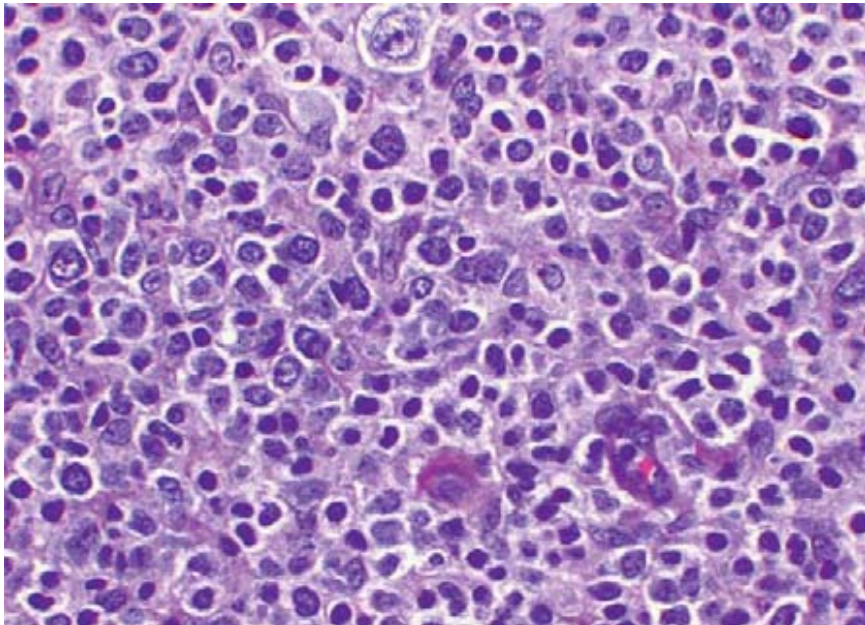


**Fig. 10-2A: Follicular lymphoma.** Back-to-back follicular nodules that replace the normal nodal tissue (Lymph node biopsy).



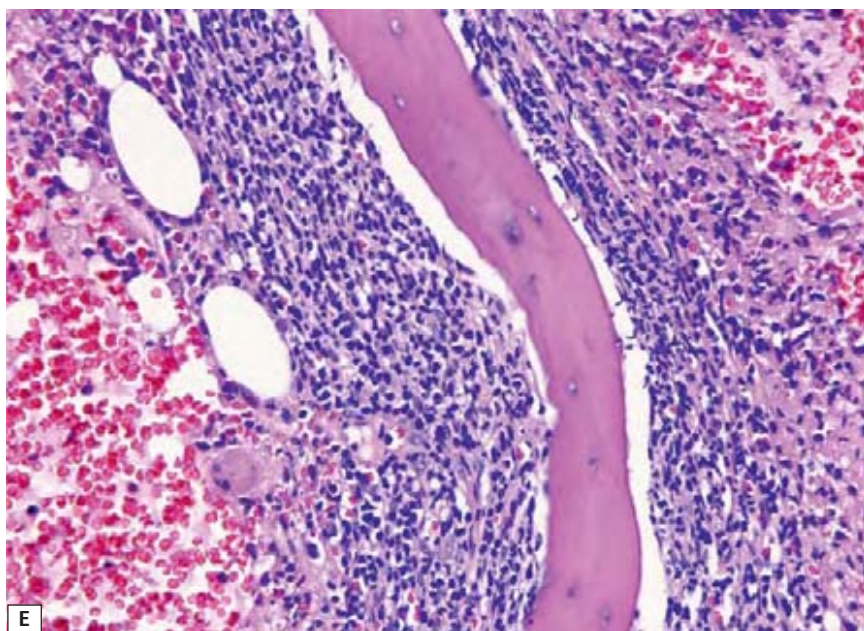
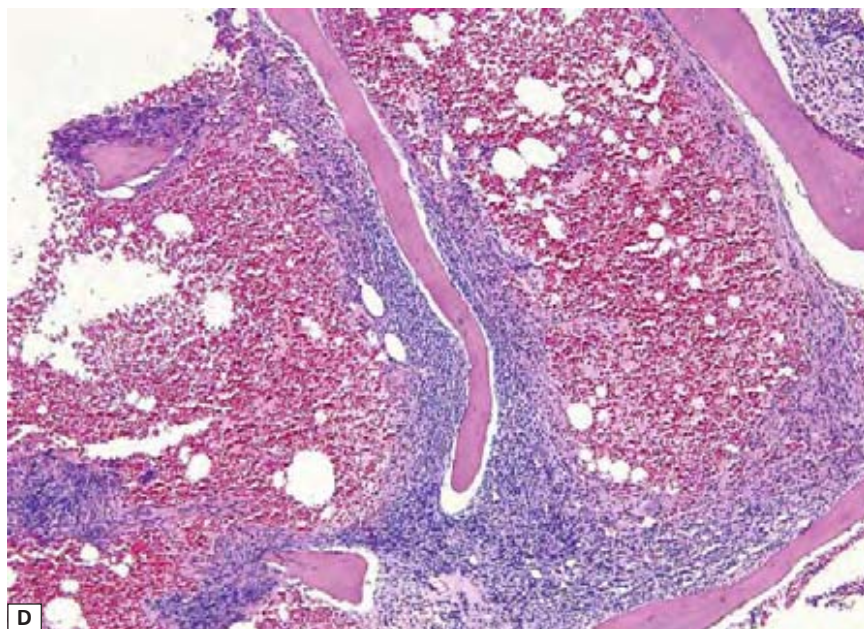


**Fig. 10-2B: Follicular lymphoma.** Low grade follicular lymphoma composed of small cleaved centrocytes with scattered centroblasts (arrows) (Lymph node biopsy).

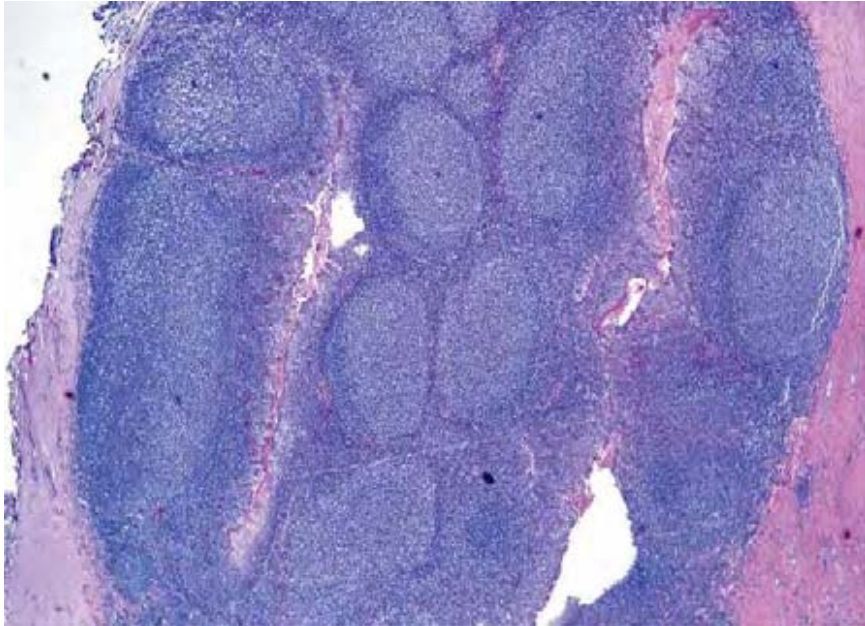


**Fig. 10-2C: Follicular lymphoma.** High grade follicular lymphoma showing a marked increase in centroblasts (Lymph node biopsy).

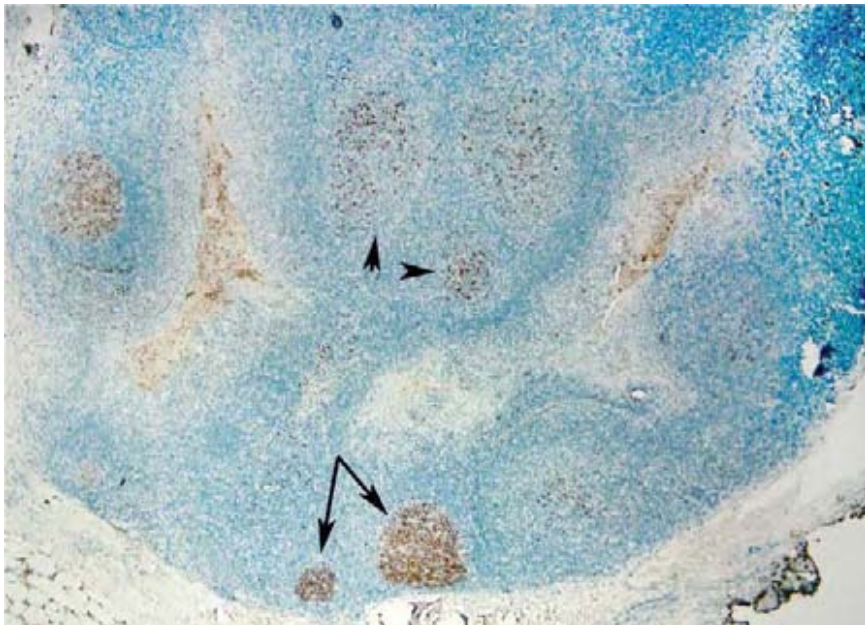




**Figs 10-2D and E: Follicular lymphoma.** The characteristic paratrabecular pattern of follicular lymphoma involving the bone marrow (Bone marrow section).

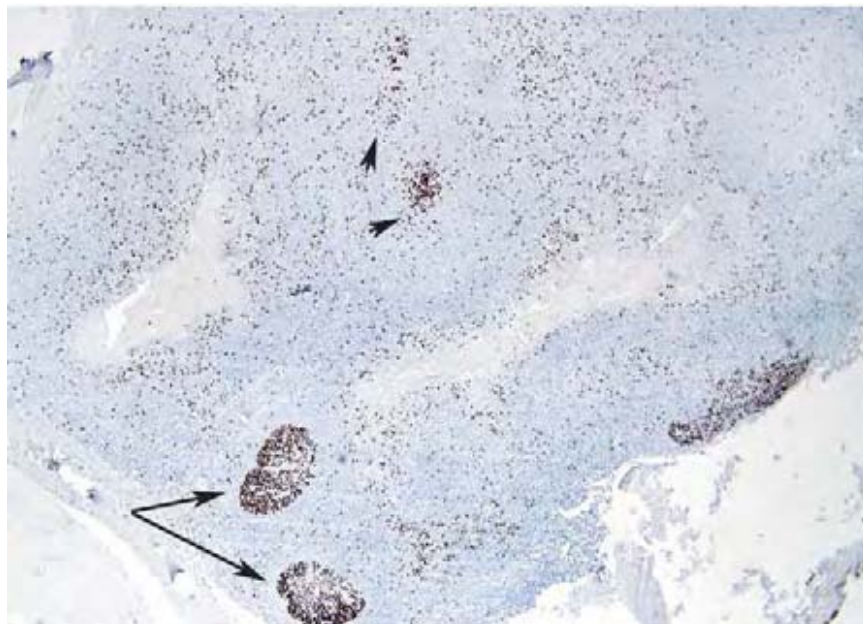


**Fig. 10-2F: Follicular lymphoma.** Partial effacement of the lymphoid follicles with some follicles still maintaining their polarity (Tonsil).



**Fig. 10-2G: Follicular lymphoma.** Immunohistochemical stain for CD10 showing the residual follicles (arrows) to have a dense staining pattern and the totally effaced follicles to have a scattered light staining pattern (arrowheads) (Tonsil).





**Fig. 10-2H: Follicular lymphoma.** Immunohistochemical stain for MIB-1 showing the residual follicles to have a dense staining pattern (arrows) and totally effaced follicles to have a scattered light staining pattern (arrowheads) (Tonsil).

### ***Mantle Cell Lymphoma***

Mantle cell lymphoma (MCL) comprises 3-10% of non-Hodgkin lymphomas. The median age is in the 6th decade of life with a male to female ratio of 2:1.

Mantle cell lymphoma is characterized by infiltration of monomorphic small to medium sized lymphocytes with irregular nuclear contours and a CCND1 translocation.

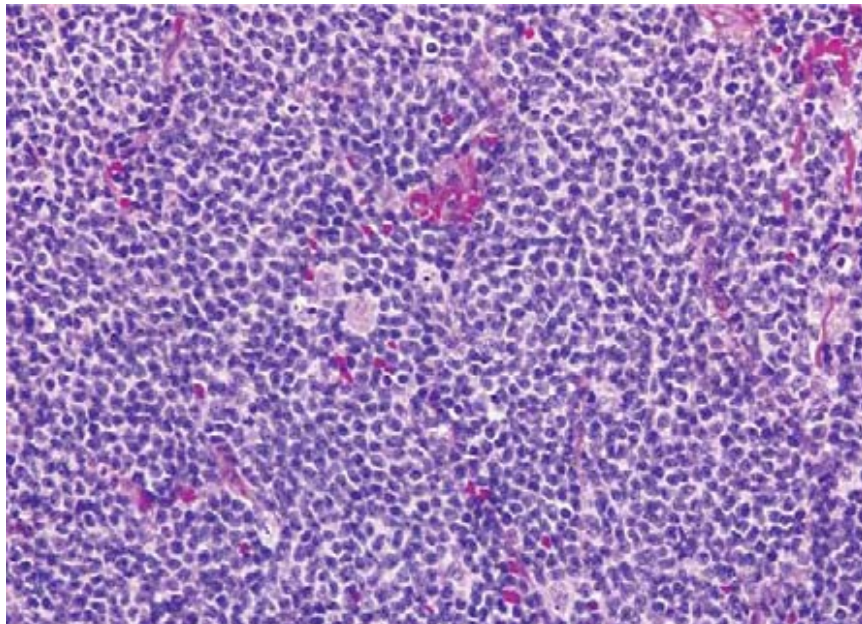
1. Peripheral blood: Lymphoma cells may be present, on a rare occasion may present as a *de novo* leukemic picture.
2. Bone marrow: May involve.
3. Lymph node: Small to medium sized tumor cells in a diffuse or nodular mantle zone pattern. The tumor cells of the blastoid variant resemble lymphoblasts with a high mitotic rate. Lymphoma may involve the spleen.
4. Flow cytometry and immunohistochemistry: Lymphoma cells are CD5, CD19, CD20, CD79a, and Cyclin D1 positive with light chain restriction. Unlike CLL/SLL, mantle cell lymphoma surface immunoglobulin expression is bright and FMC7 is positive. CD10, CD11c, CD23, CD25, and CD103 are negative.

5. Cytogenetic and molecular studies: Characteristic **t(11;14)(q13;q32)**, resulting in the overexpression of the CCND1 (PRAD1) gene.  
Other genetic abnormalities may also present:
  - Del 13q14 (40-50% of cases)
  - Del 7p13 (TP53, 20-45% of cases)
  - Del 11q23 (ATM, 20-60% of cases)
  - Del 9p21 (CDKN2A/INK4a, 20-30% of cases)
  - Tetraploidy (common in pleomorphic and blastoid variants)
  - +8q24 and t(8)(q24) (common in pleomorphic and blastoid variants).
6. The blastic variant of MCL may resemble diffuse large B-cell lymphoma but cyclin D1 is positive and the **t(11;14)** translocation is diagnostic for mantle cell lymphoma (Figs 10-3A to E).

### Diffuse Large B-cell Lymphoma (DLBCL)

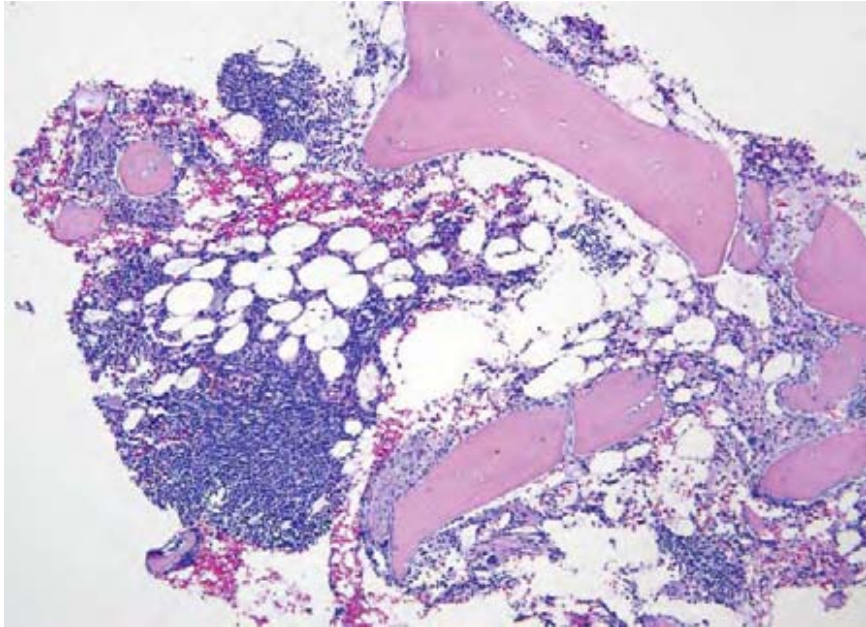
Diffuse large B-cell lymphoma is the most common type of lymphoid malignancy, accounting for approximately 40% of all the non-Hodgkin lymphomas.

Diffuse large B-cell lymphoma is a diffuse proliferation of large neoplastic B-lymphocytes. Diffuse large B-cell lymphoma either occurs *de novo* or

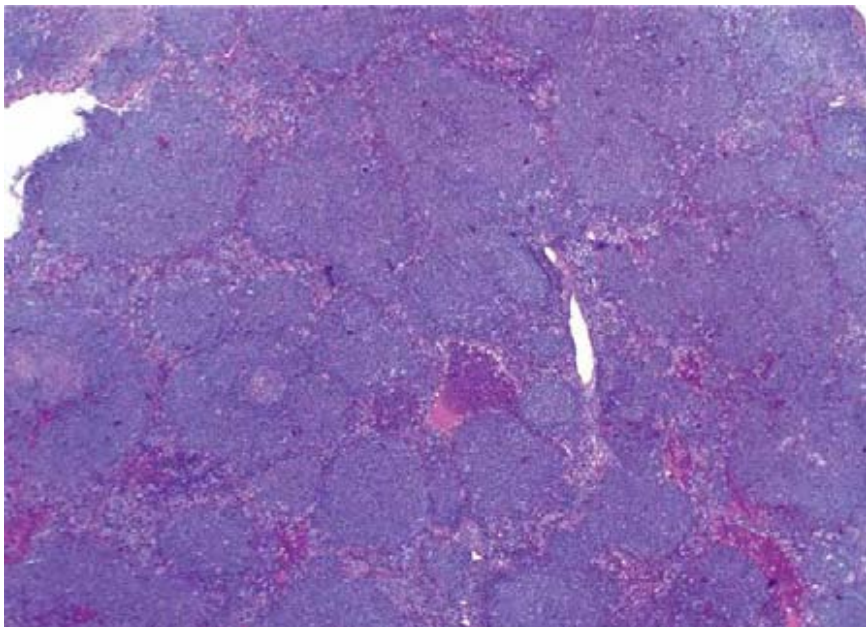


**Fig. 10-3A: Mantle cell lymphoma.** The tumor cells are small to medium size with irregular nuclei (Lymph node biopsy).

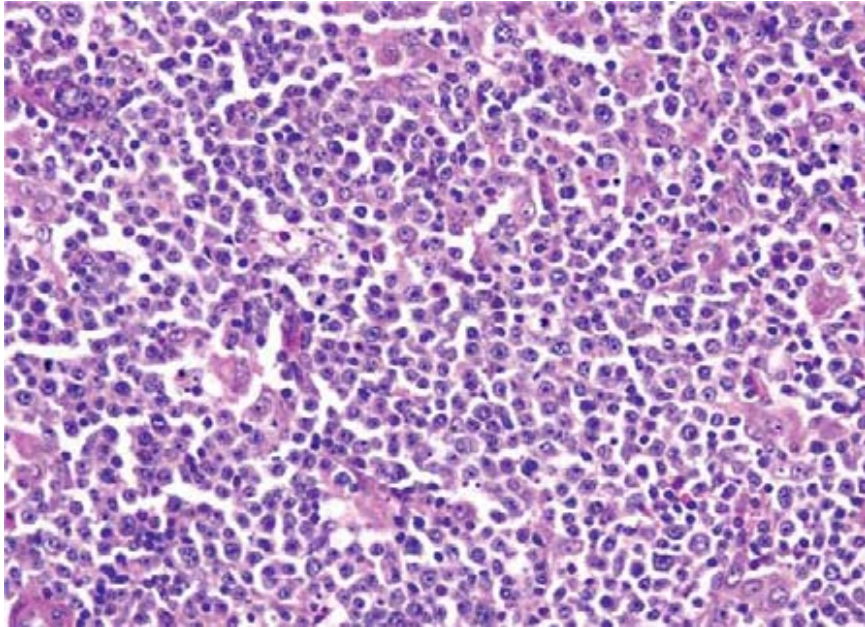




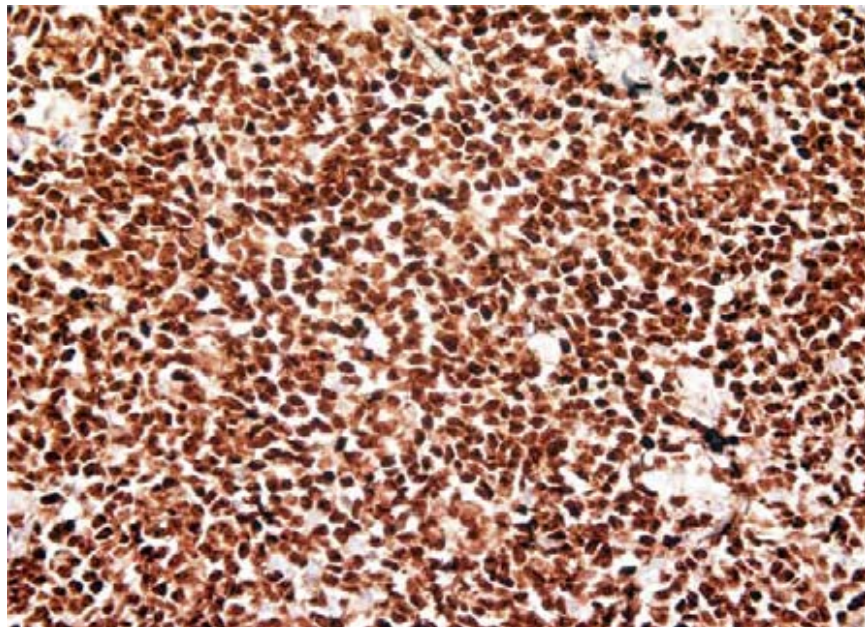
**Fig. 10-3B: Mantle cell lymphoma.** Involvement of the bone marrow in a nodular pattern. A paratrabecular pattern (like follicular lymphoma) is extremely rare (Bone marrow section).



**Fig. 10-3C: Mantle cell lymphoma.** The nodular variant of mantle cell lymphoma (Bone marrow section).



**Fig. 10-3D: Mantle cell lymphoma.** The blastoid variant of mantle cell lymphoma has a high mitotic rate and tumor cells resembling lymphoblasts (Bone marrow section).



**Fig. 10-3E: Mantle cell lymphoma.** Immunohistochemical stain for cyclin D1 is diagnostic for mantle cell lymphoma (nuclear staining pattern) (Lymph node biopsy).



from transformed low-grade B-cell lymphoma. The size of a nucleus in large lymphoma cells is equal to or exceeding the **nucleus of a macrophage** or more than **twice the size** of a normal lymphocytes.

***Diffuse Large B-cell Lymphoma (DLBCL),  
Not Otherwise Specified***

1. Peripheral blood: Involvement is uncommon except in intravascular large B-cell lymphoma.
2. Bone marrow: May involve.
3. Flow cytometry and immunohistochemistry: Lymphoma cells express B-cell related markers (CD19, CD20, CD22, and CD79a) with light chain restriction. Flow cytometry may be negative due to the size of lymphoma cells or necrosis of the specimen.
4. Cytogenetic and molecular studies: Complex cytogenetic abnormalities.
  - IgH gene rearrangement is positive.
  - Translocations may be present in DLBCL:
    - BCL2 (20-30% of cases)
    - BCL6 (3q27, up to 30% of cases, most common in DLBCL)
    - cMYC (up to 10% of cases).
5. Differential diagnosis:
  - Carcinoma (especially nasopharyngeal carcinoma)
  - Germ cell tumor
  - Burkitt lymphoma variants
  - Blastoid mantle cell lymphoma
  - Granulocytic sarcoma.
6. DLBCL gene profiling variants
  - Germinal center B-cell (GCB)
  - Activated B-cell (ABC) or non-germinal B-cell (has adverse prognosis)

Hans et al developed an immunohistochemical algorithm with approximately 80% concordance with the gene expression profiling (GEP) classification of diffuse large B-cell lymphoma (DLBCL) into the germinal center B-cell-like (GCB) and activated B-cell-like (ABC or non-germinal center type) subtypes. This immunohistochemical algorithm is based on three antigens (CD10, Bcl-6, and multiple myeloma-1/interferon regulatory factor-4 [MUM1/IRF4]). CD10 and Bcl-6 are expressed by germinal center B-cells, while MUM1/IRF4 is expressed on B cells that are on the verge of exiting or have exited the germinal center. If CD10 is positive, DLBCL is classified as GCB subtype. If CD10 is negative but BCL6 is positive and MUM1 is negative, DLBCL is classified as GCB subtype. If both CD10 and

BCL6 are negative or CD10 is negative but both BCL6 and MUM1 are positive, DLBCL is classified as ABC subtype (Table 10-3).

**TABLE 10-3****Use of immunohistochemical stains to classify GCB or non-GCB\***

GCB	ABC or non-GCB
CD10+ or CD10-, BCL6+, MUM1-	CD10 and MUM1+ (>30% positivity) Either BCL6+ or BCL6-

\*IHC profiling is controversial and does not have a rule in current clinical practice. Rituximab+ CHOP treatment regimen may eliminate the difference between these two groups.

### ***DLBCL Morphological Variants***

1. **Centroblastic:** Most common type, medium to large sized vesicular nuclei with fine chromatin and 2-5 nucleoli adjacent to the nuclear membrane.
2. **Immunoblastic:** Rare variant, 90% of the cells are immunoblasts with a single centrally located nucleolus.
3. **Anaplastic:** Composed of large pleomorphic cells that express B-cell markers. Many of the cases are CD30 positive, but ALK-1 is negative (Figs 10-4A to E).

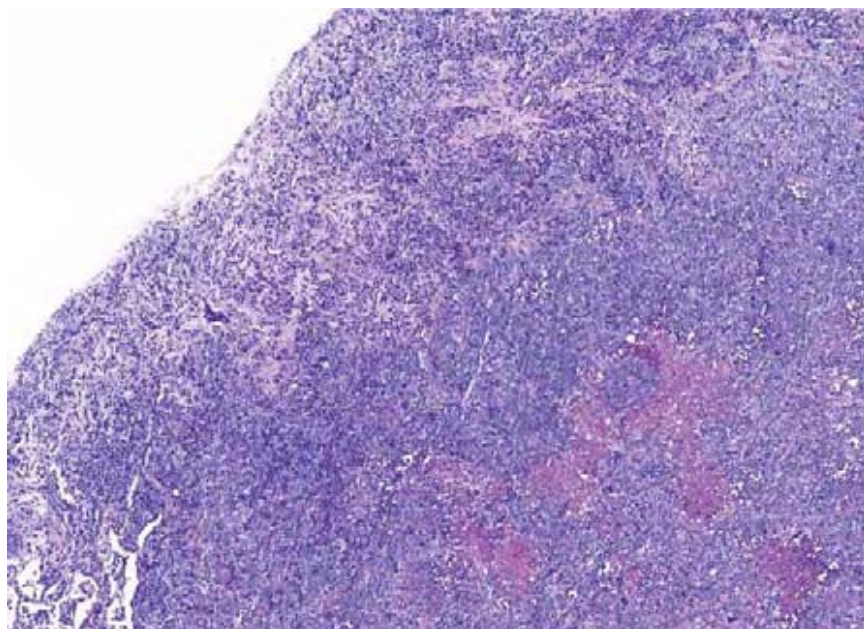
### ***DLBCL Subtypes***

1. **T-cell/histocyte rich large B-cell lymphoma (THRLBCL)** is a lymphoma in which large neoplastic B-cells comprise less than 10% of the total infiltrating lymphoid cells. A background of T-cells with or without histiocytes surrounds these large neoplastic cells. The differential diagnosis is nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) (Table 10-4).

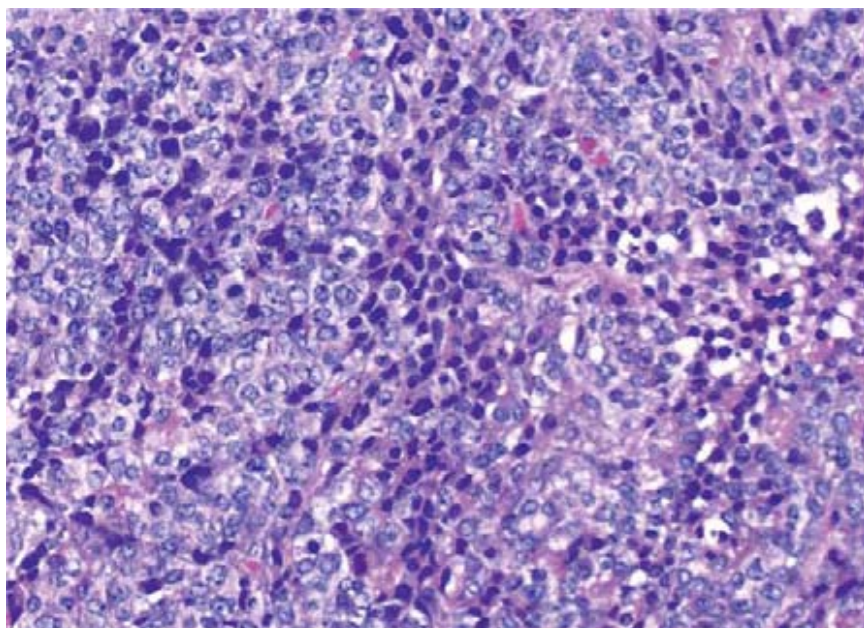
**TABLE 10-4****Comparison of THRLBCL and NLPHL**

	THRLBCL	NLPHL
Large cell Background T-cells Dendritic cells Distribution of large B-cells	Variable morphology Mostly CD8+ Absent (CD21-) Scattered all over the tissue (total number of large B-cells are <10% of total tissue)	Popcorn cells Mostly CD4+ Present (CD21+) Clustered within small B-cell nodules

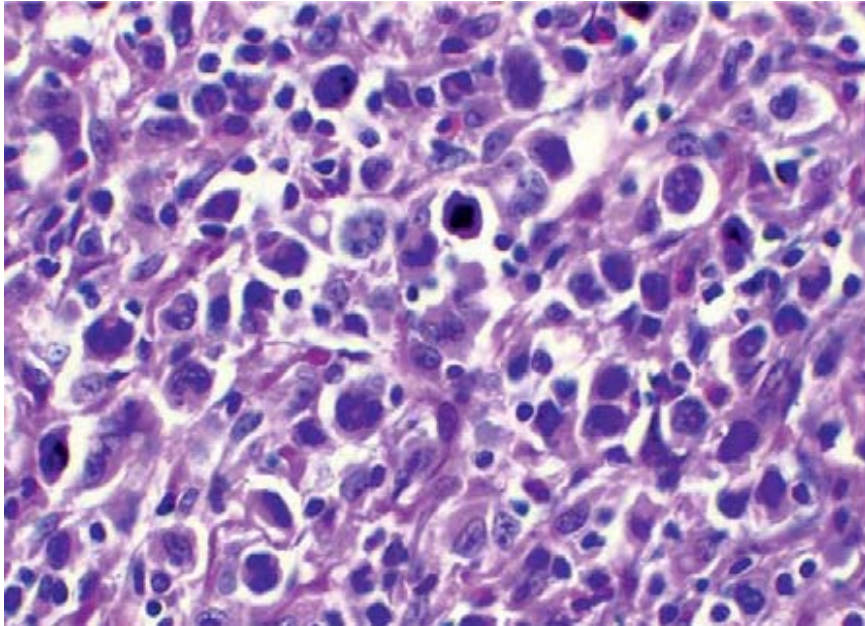




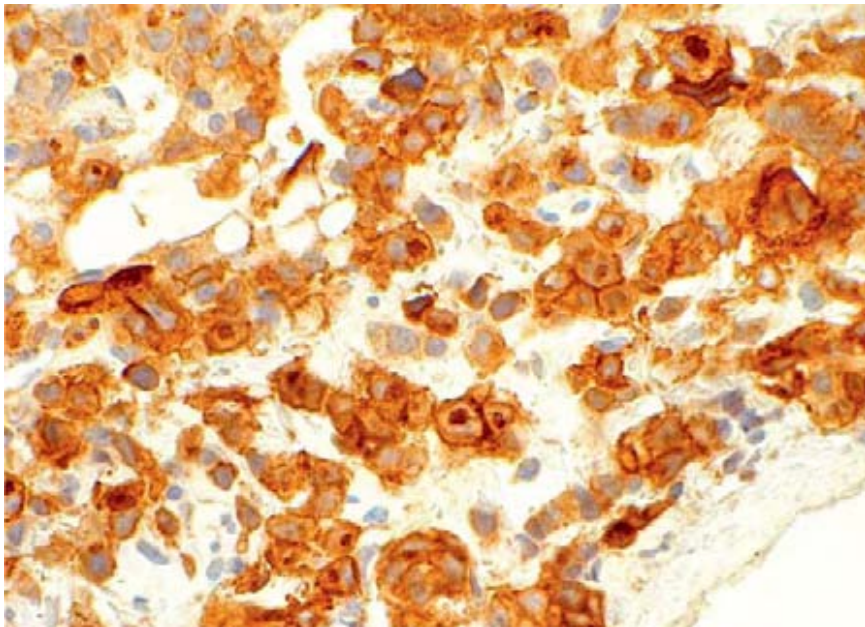
**Fig. 10-4A:** Diffuse large B-cell lymphoma showing sheets of large cells with focal necrosis (Lung section).



**Fig. 10-4B:** Diffuse large B-cell lymphoma. The tumor cells are larger than normal lymphocytes and show vesicular nuclear chromatin (Lung section).

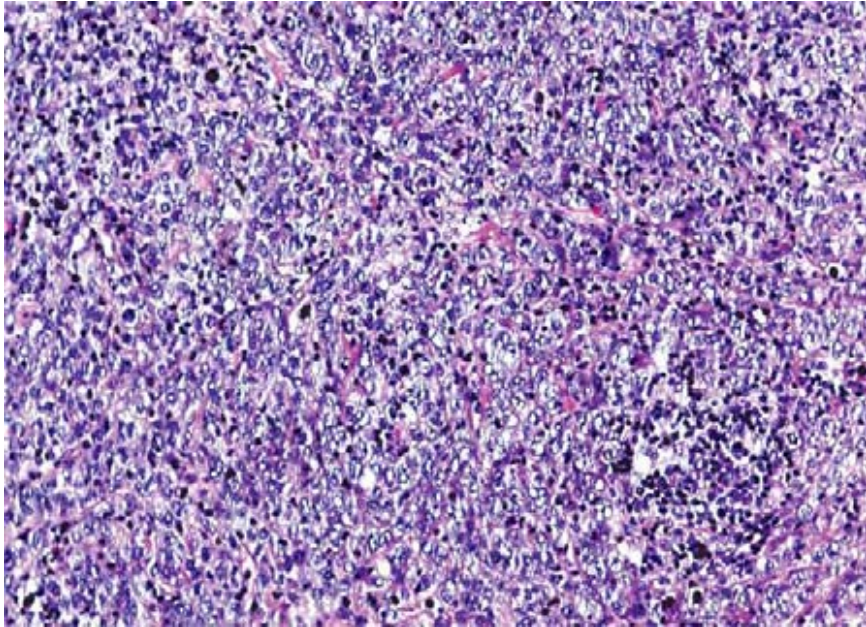


**Fig. 10-4C: Diffuse large B-cell lymphoma, anaplastic variant.**  
Large pleomorphic cells (Lung section).



**Fig. 10-4D: Diffuse large B-cell lymphoma, anaplastic variant.** Immunohistochemical stain for CD30 highlights the large, pleomorphic tumor cells (Lung section).





**Fig. 10-4E: Diffuse large B-cell lymphoma centroblastic variant.** The tumor cells are medium to large in size with vesicular nuclear chromatin, and 2-5 peripheral located nucleoli (Lymph node biopsy).

2. **Primary diffuse large B-cell lymphoma of the CNS** comprises <1% of non-Hodgkin lymphoma and is the **most common primary lymphoma of the CNS**. Intracerebral and intraocular locations are most common.
3. **Primary cutaneous DLBCL, leg type** occurs in elderly women, most commonly affects the lower leg (85-90%). The lymphoma cells express CD20, CD79a, BCL2, MUM1, and BCL6 (most of the cases). They are CD10 negative.
4. **EBV-positive DLBCL of the elderly** is an EBV+ clonal B-cell lymphoma that occurs in patients >50 years old without any known immunodeficiency or prior lymphoma. Other well-defined disorders that may be EBV+ are excluded from this category.
5. **DLBCL associated with chronic inflammation** is a lymphoma occurring in the context of long-standing chronic inflammation that shows an association with EBV, such as pyothorax-associated lymphoma (PAL). Most cases involve the pleural cavity or bone marrow.
6. **Lymphomatoid granulomatosis (LYG)** is a rare disorder and is characterized by angioinvasion, focal necrosis and extranodal site involvement (lung >90%, brain 26%, kidney 29%, liver 29%, skin

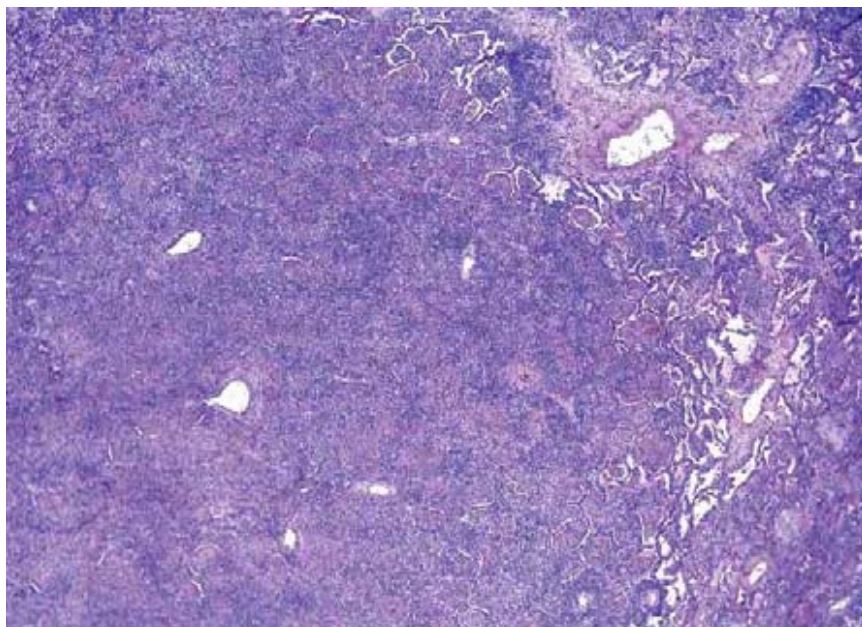
25-50%). It usually presents in adult life, but may be seen in children with immunodeficiency disorders. The male to female ratio is  $\geq 2:1$ . The clinical course is aggressive in most patients. Some patients may follow a waxing and waning clinical course with rare spontaneous remission occurring without therapy.

Histology shows predominantly reactive T-cells with scattered atypical large EBV+ B-cells (CD20+, variable CD30+ and CD15-).

The grading of lymphomatoid granulomatosis relates to the proportion of EBV positive cells to reactive T-cells.

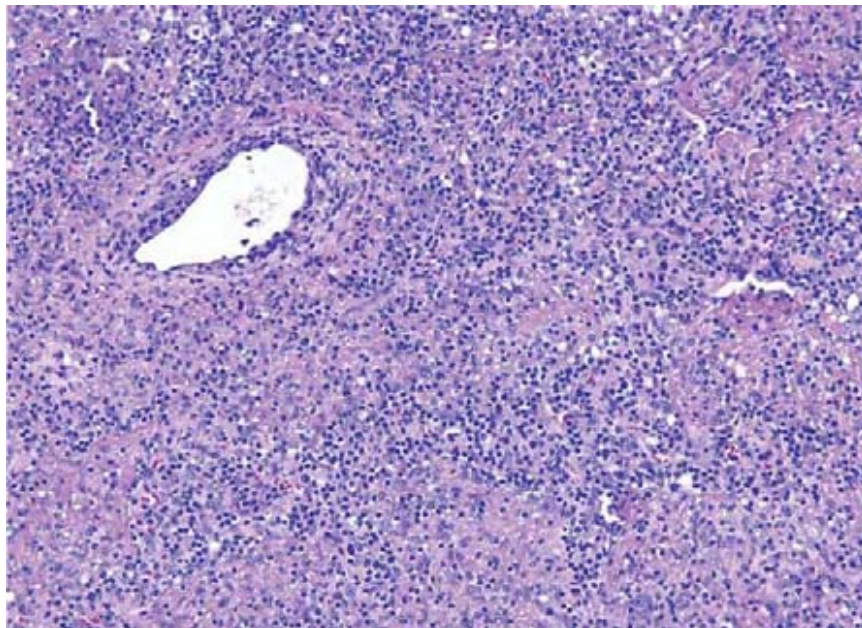
- Grade 1 has <5% EBV+ cells/HPF, large EBV transformed cells are absent or rare and necrosis is usually focal.
- Grade 2 has 5-20 EBV+ cells/HPF, occasional or small clusters of large EBV transformed cells may be seen, and necrosis is common.
- Grade 3 has >50 EBV+ cells/HPF an inflammatory background is still present but contains large atypical cells. Necrosis is extensive (Figs 10-5A to E).

7. **ALK positive DLBCL** shows a sinusoidal growth pattern. These tumor cells are CD30-, ALK-1+ (cytoplasmic staining pattern) but lack the t(2;5) translocation. There is also strong expression of EMA and plasma cell markers (CD138, VS38). Lymphoid lineage markers CD3, CD20 and CD79a are usually negative, and the expression of CD45 is usually

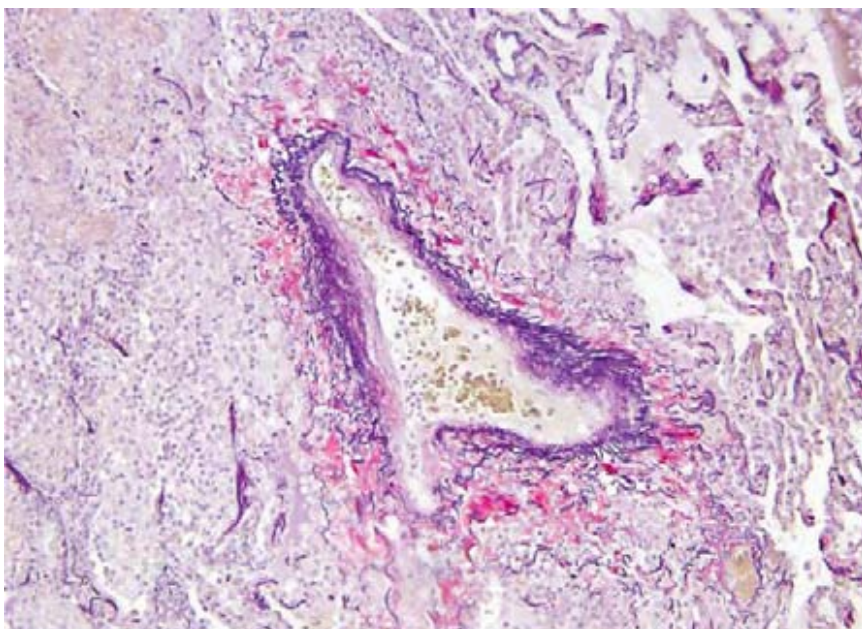


**Fig. 10-5A: Lymphomatoid granulomatosis.** Lung section showing a pleomorphic lymphoid infiltration and granulomas.

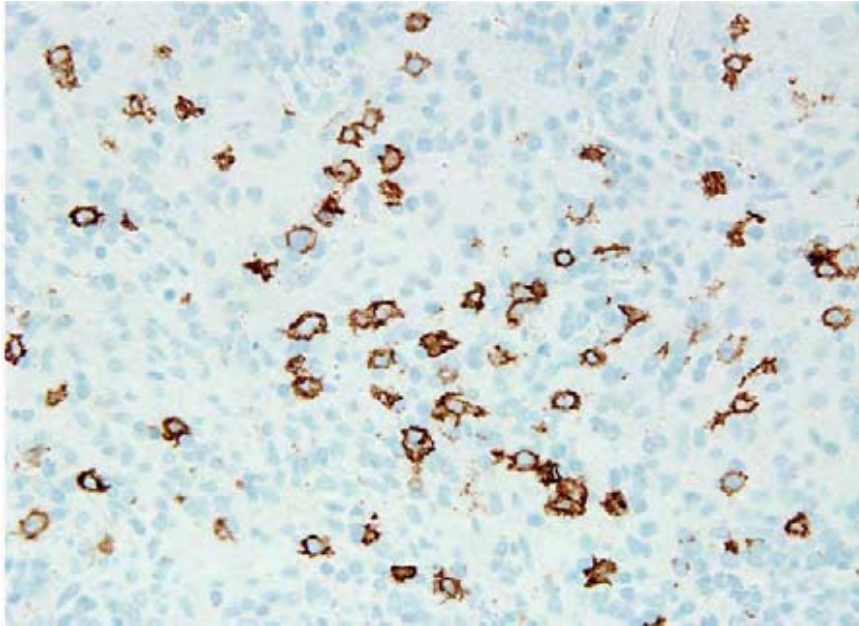




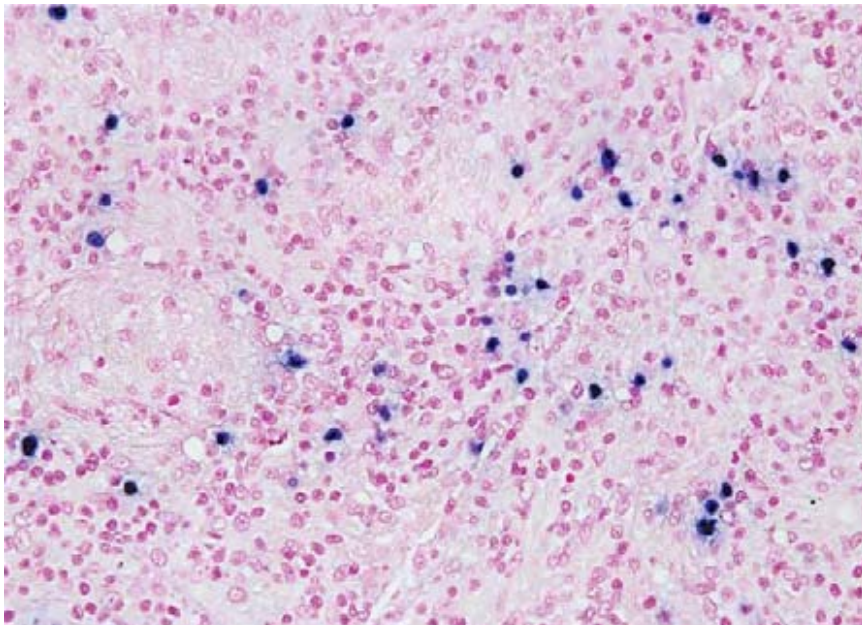
**Fig. 10-5B: Lymphomatoid granulomatosis.** Lung section showing an angiocentric and angiodestructive pleomorphic lymphoid infiltration.



**Fig. 10-5C: Lymphomatoid granulomatosis.** Verhoeff-Van Gieson (VVG) staining for elastic fibers highlights the disruption of vessel's elastic wall (Lung section).

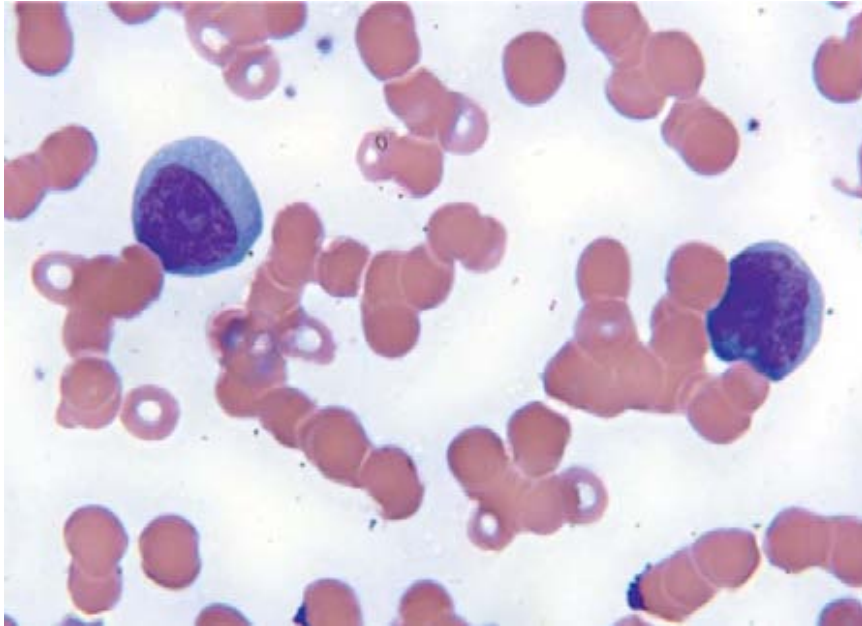


**Fig. 10-5D: Lymphomatoid granulomatosis.** Immunohistochemical stain for CD20 highlighting scattered, large CD20 positive cells (Lung section).

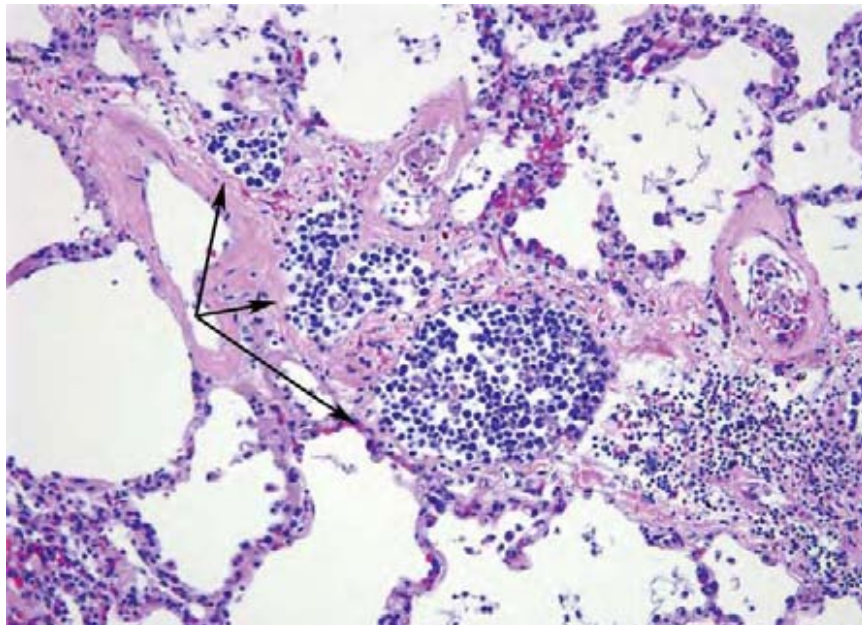


**Fig. 10-5E: Lymphomatoid granulomatosis.** In situ hybridization for EBER highlights the CD20 positive cells which are infected by Epstein-Barr virus (Lung section).

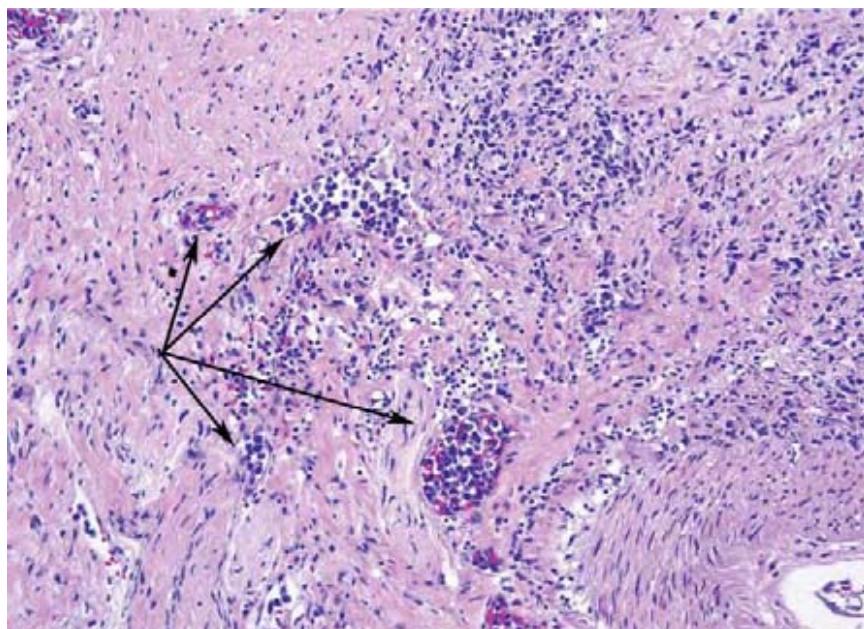




**Fig. 10-6A: Intravascular large B-cell lymphoma.** Peripheral blood smear showing large neoplastic cells with a centrally located nucleoli.



**Fig. 10-6B: Intravascular large B-cell lymphoma.** Large neoplastic cells located in the microvasculature and large vessels (arrows) (Autopsy lung section).

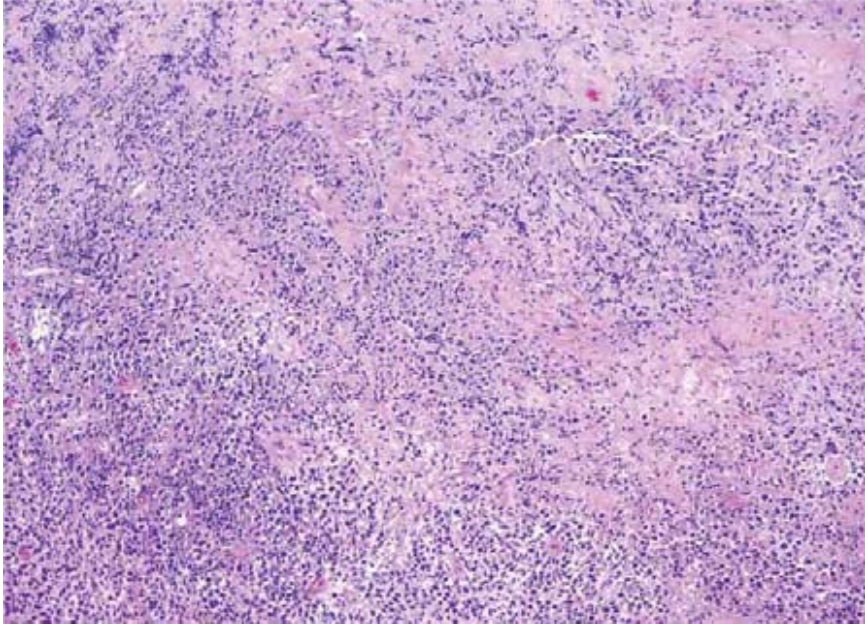


**Fig. 10-6C: Intravascular large B-cell lymphoma.** Large neoplastic cells located in the microvasculature and large vessels (arrows) (Autopsy ovary section).

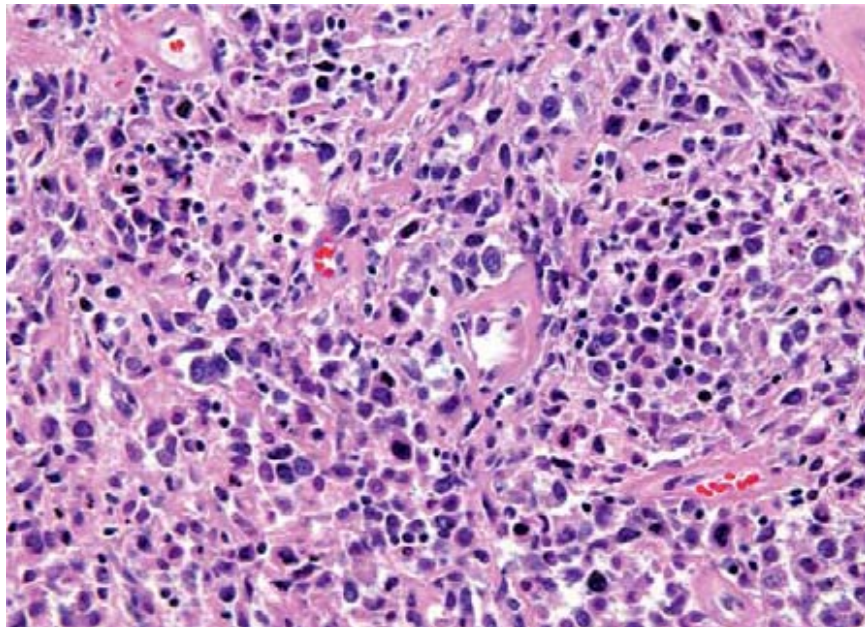
weak. This entity should be distinguished from CD30+ ALK+ T/null anaplastic large cell lymphoma, DLBCL with a sinusoidal growth pattern and ALK-1 negative immunoblastic/plasmablastic lymphoma.

8. **Intravascular large B-cell lymphoma** is a rare disorder showing intravascular lymphoma cells confined to the lumen of blood vessels. The most common site is the central nervous system. Patients initially present with hepatosplenomegaly, but lymphadenopathy is rare. Tumor cells express B-cell markers. CD5 and CD10 positivity are seen in 38% and 13% of the cases respectively. Almost all CD10 negative cases are IRF4/MUM1 positive (Figs 10-6A to C).
9. **Primary mediastinal (thymic) large B-cell lymphoma** is most commonly seen in young adults. Females are more commonly affected than males. This subtype of large B-cell lymphoma is confined to the mediastinum and thought to be derived from the small native population of B-cells that resides within thymic tissue. Morphology shows centroblastic, multilobated cells in a sclerotic stroma. The lymphoma cells express pan B-cell markers and are **CD30+** in 80% of the cases. Cytogenetic and molecular studies:
  - 9p24 (75%)
  - 2p15 (50%)
  - Xp11.4-21 and Xq24-26 (33%) (Figs 10-7A and B).





**Fig. 10-7A: Primary mediastinal large B-cell lymphoma.** This subtype of diffuse large B-cell lymphoma is often characterized by sclerosis or fibrosis, which results in small packets of neoplastic cells (Mediastinal biopsy).



**Fig. 10-7B: Primary mediastinal large B-cell lymphoma.** Individual tumor cells often have a polylobulated nuclear appearance (Mediastinal biopsy).

- 10. Plasmablastic lymphoma** is uncommon. It is mostly seen in HIV patients and has a male predominance. The tumor cells are often positive for CD79a (50-85%), MUM1 and CD138, and negative or weakly positive for CD45, CD20 and PAX-5. EMA and CD30 are frequently positive. EBV-EBER is positive in 60-75% of the cases. EBV-LMP1 is usually negative. Plasmacytoma or myeloma should be ruled out if these tumor cells are also CD56 positive (Figs 10-8A and B).
- 11. Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease** is a large B-cell lymphoma arising from human herpes virus associated multicentric Castleman's disease. Lymphoma cells strongly express cIgM with  $\lambda$  light chain restriction and CD20+/-, CD79a-, CD138-, CD38-/-, HHV8+, and EBER-.
- 12. Primary effusion lymphoma** is commonly seen in AIDS. Lymphoma cells are usually CD45 positive but lack B- or T-cell markers. Surface and cytoplasmic immunoglobulin is absent. Activation related markers and plasma cell-related antigens may be positive (HLA-DR, CD30, CD38, Vs38c, CD138, and EMA). Lymphoma cells may be positive for HHV8 or EBER.

***B-cell Lymphoma, Unclassifiable with Features Intermediate between DLBCL and Burkitt Lymphoma***

These are aggressive lymphomas, which have morphologic and genetic features of both DLBCL and Burkitt lymphoma. Some of these cases were previously classified as Burkitt-like lymphoma.

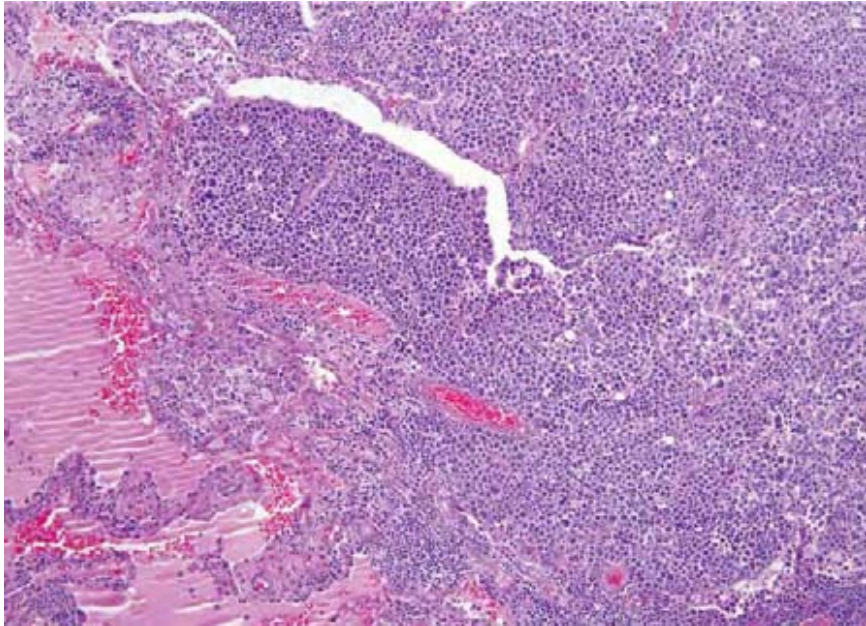
The diagnosis of this unclassifiable lymphoma should not be made in cases of typical DLBCL with a MYC rearrangement or typical Burkitt lymphoma in which a CMY rearrangement cannot be demonstrated.

Some transformed follicular lymphoma or a typical Burkitt's lymphoma but with a strong BCL2 expression may fall into this category.

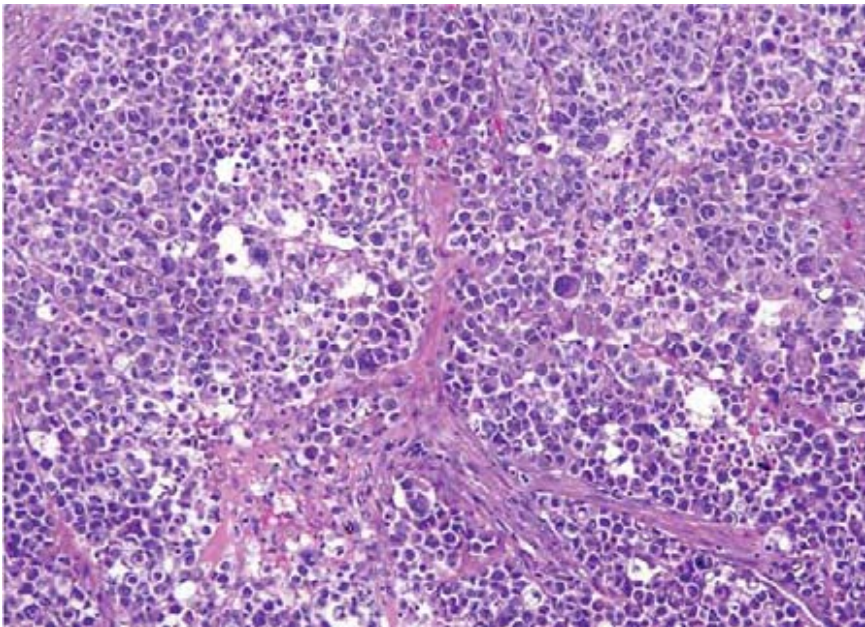
***B-cell Lymphoma, Unclassifiable with Features Intermediate between DLBCL and Classical Hodgkin Lymphoma (CHL)***

These B-cell lymphomas show an overlap between features of DLBCL and CHL. The large B-cells are CD30+, CD15+ (majority), CD20+ (frequently), and CD79a+. Transcription factors PAX-5, OCT-2, and BOB.1 are usually positive.





**Fig. 10-8A: Plasmablastic lymphoma.** This subtype of diffuse large B-cell lymphoma shows plasmablastic features, occasionally plasma cells can be identified (Lung section).



**Fig. 10-8B: Plasmablastic lymphoma.** Lung section showing large pleomorphic cells with eosinophilic cytoplasm. These tumor cells are positive for CD79a and CD138. MIB-1 staining is nearly 100% (Lung section).

## Burkitt Lymphoma

Burkitt lymphoma (BL) is one of the highly aggressive lymphomas that is associated with Epstein-Barr virus and has a specific chromosomal translocation involving chromosome 8. Burkitt lymphoma presents in three clinically distinct forms: endemic, sporadic and immunodeficiency associated.

Burkitt lymphoma patients have a high risk for CNS system involvement. Other extranodal sites are frequently involved.

With intensive chemotherapeutic regimens, the majority of patients are cured.

1. Three variants:
  - **Endemic:** occur in equatorial Africa and Papua New Guinea, peak incidence at 4-7 years old.
  - **Sporadic:** throughout the world, mainly children and young adults.
  - **Immunodeficiency-associated:** primarily seen in HIV patients.
2. Peripheral blood: Involvement is uncommon.
3. Bone marrow: Frequently involved.
4. Lymph node: The morphology shows architectural effacement by monomorphic, medium sized cells in a “starry sky” pattern that characterizes Burkitt lymphoma.
5. Flow cytometry and immunohistochemistry: Lymphoma cells express pan B-cell markers (CD19, CD20 and CD79a), CD10, CD43, and BCL6. BCL2 and TdT are negative.
6. Cytogenetic and molecular studies: Characteristic translocation of **8q24 (MYC) to 14q32 (IgH)** or less commonly to **22q11 (Kappa light chain) or 2p12 (Lambda light chain)** (Figs 10-9A and B).
  - t(8;14)
  - t(8;22)
  - t(2;8)

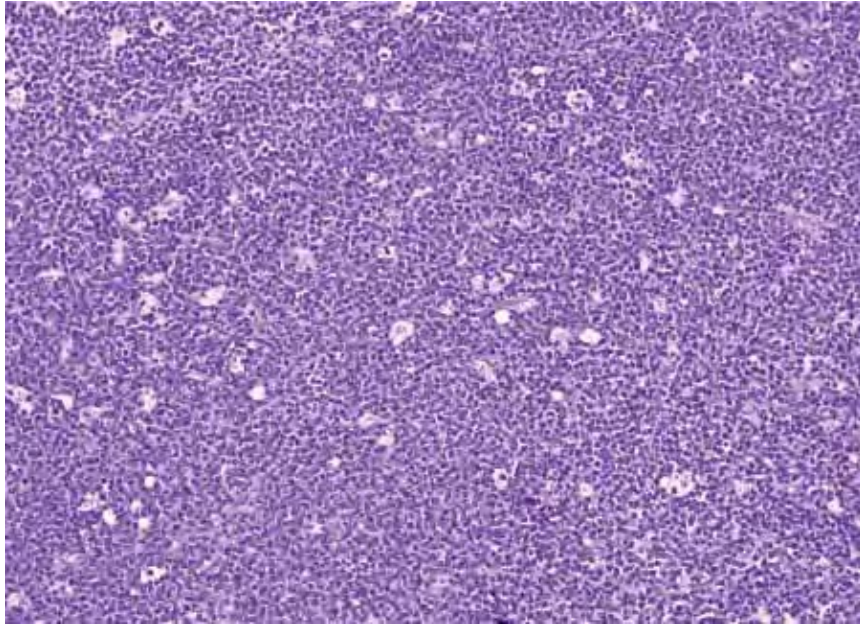
## Mature T-cell and Natural Killer (NK) Cell Neoplasm

### *Extranodal NK/T-cell Lymphoma, Nasal Type*

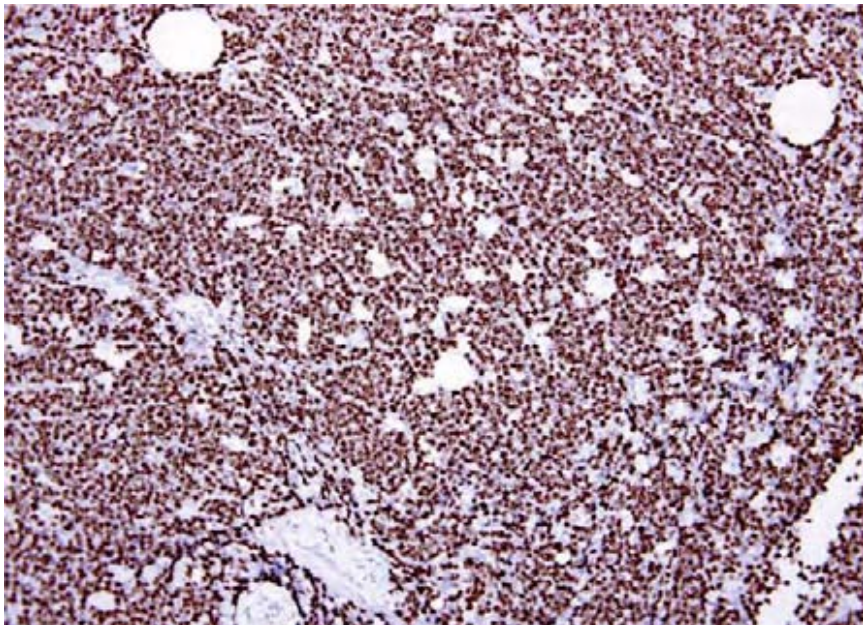
Extranodal NK/T-cell lymphoma is an aggressive lymphoma characterized by prominent necrosis, vascular damage and destruction, and is associated with EBV infection. The most common clinical presentation is a destructive midline facial tumor.

1. Nasal and non-nasal type NK/T-cell lymphomas have the same morphologic, phenotypic and molecular features.





**Fig. 10-9A: Burkitt lymphoma.** The normal lymph node architecture is totally effaced by sheets of medium sized lymphoblasts and scattered macrophages ("starry sky" appearance) (Lymph node biopsy).



**Fig. 10-9B: Burkitt lymphoma.** Immunohistochemical stain for MIB-1 showing a very high proliferation rate of 100%. These tumor cells are also positive for CD10 and BCL6, but negative for BCL2 (Lymph node biopsy).

2. Morphology: Shows angiocentric and angioinvasive lymphocytes, immunoblasts, plasma cells, and less frequently, eosinophils and histiocytes. The extensive necrosis is chemokine mediated.
3. Immunophenotype: Lymphoma cells are CD2+, CD5+, CD7+, TIA+, granzyme B+, perforin+, CD4+ (common) or CD8+, and frequently CD56+.
4. Cytogenetic and molecular studies: TCR and IgH gene rearrangements are usually negative. In rare cases, TCR gene rearrangement may show clonality.

### ***Enteropathy Associated T-cell Lymphoma (EATL)***

Enteropathy associated T-cell lymphoma is a rare T-cell lymphoma, which is often associated with gluten sensitive enteropathy, and usually involves the jejunum or ileum. Involvement of stomach, colon and locations outside of the gastrointestinal tract are rare. The neoplastic cells are a mix of small, medium and large-sized lymphocytes with an immunophenotype of CD3+, CD7+, CD8+/-, and CD103+. CD4, CD5 and CD56 are usually negative.

Type II EATL accounts for 10-20% of the EATL cases. This variant is composed of monomorphic medium-sized cells and has an immunophenotype of CD3+, CD8+ and CD56+. Type II EATL may occur sporadically without the associated risk factor of celiac disease.

Cytogenetic and molecular studies:

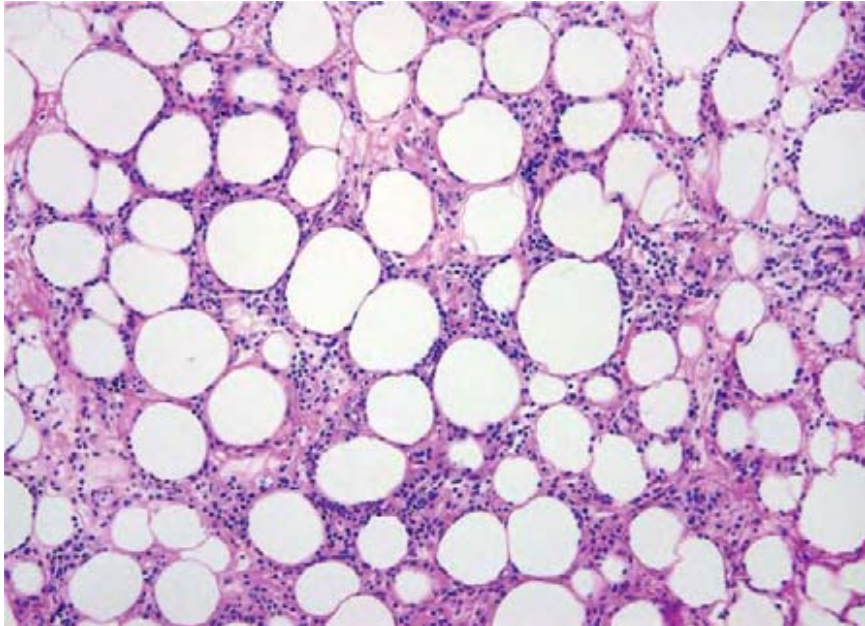
- +9q31.3 or -16q12.1 (common for both types)
- +1q32.2-q41 or +5q34-q35.2 (less common in type II)
- +8q24 (MYC) (less common in type I)
- TCR gene rearrangement is positive.

### ***Subcutaneous Panniculitis-like T-cell Lymphoma***

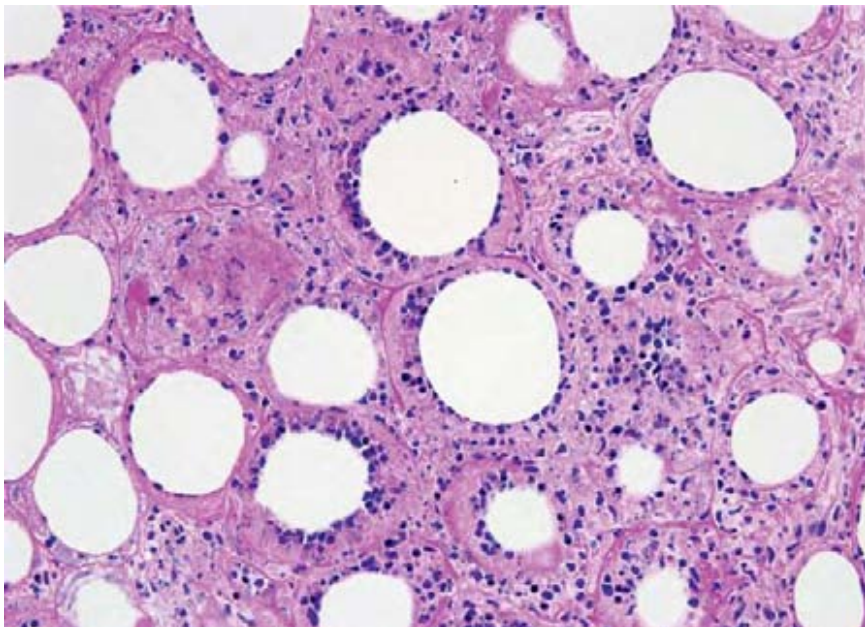
Patients typically present with subcutaneous nodules on the extremities. Most cases are associated with hemophagocytic syndrome.

1. Morphology: The lesion is usually confined to the subcutis. The neoplastic cells are monotonous and encircle fat lobules in a lace-like pattern. This **rimming of fat cells by neoplastic T-cells is characteristic** of this lesion.
2. Immunophenotype: CD8+,  $\beta$ F1+, TIA+, and perforin+. EBV is consistently negative.
3. Cytogenetic and molecular studies: No specific cytogenetic abnormality. TCR gene rearrangement is positive (Figs 10-10A and B).





**Fig. 10-10A: Subcutaneous panniculitis-like T-cell lymphoma.** Skin biopsy showing characteristic rimming of fat cells by neoplastic T-cells (*Courtesy: Dr Garth Fraga, University of Kansas Medical Center, Kansas City, KS, USA*).



**Fig. 10-10B: Subcutaneous panniculitis-like T-cell lymphoma.** Skin biopsy (high magnification) showing characteristic rimming of fat cells by neoplastic T-cells (*Courtesy: Dr Garth Fraga, University of Kansas Medical Center, Kansas City, KS, USA*).

### ***Mycosis Fungoides***

Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma and accounts for near 50% of all cutaneous lymphomas. Patients have an indolent clinical course with slow progression from patches to plaques and eventually tumor stage that spans over years.

1. Morphology: **The neoplastic cells have cerebriform-shaped nuclei, are located in the upper dermis,** and frequently infiltrate the epidermis (epidermotropism). A small aggregate of lymphocytes (Pautrier microabscesses) may also be seen in the epidermis. Involvement of peripheral blood and lymph nodes may be present.
2. Immunophenotype: CD2+, CD3+, CD4+, CD5+ and CD8-/+.
3. Cytogenetic and molecular studies: No specific cytogenetic abnormality, complex cytogenetic karyotype (especially in advanced stage of disease). TCR gene rearrangement is positive (Figs 10-11A to C).

### ***Primary Cutaneous CD30+ T-cell Lymphoproliferative Disorders***

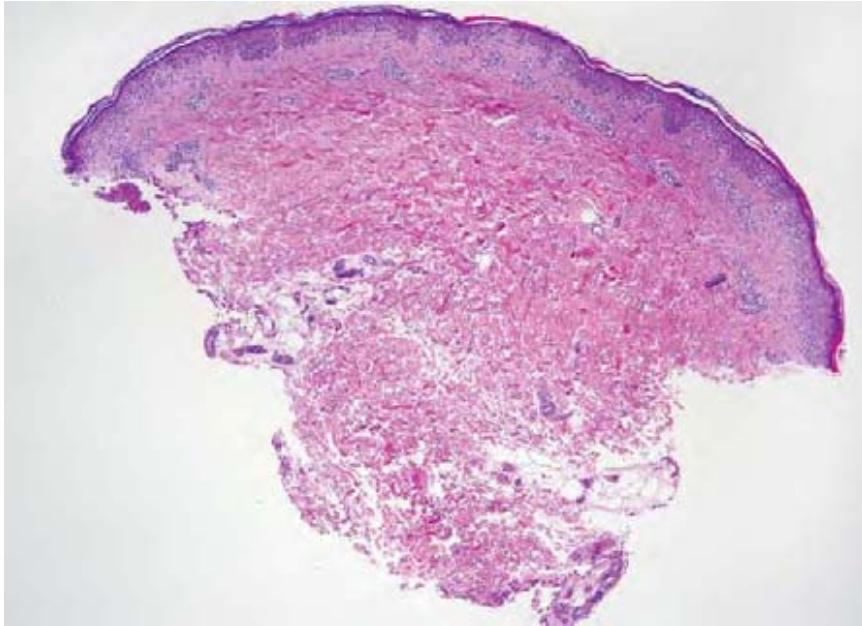
Primary cutaneous anaplastic large cell lymphoma and lymphomatoid papulosis represent a continuous clinical and histopathologic spectrum of related diseases. Lymphomatoid papulosis manifests as multiple papular lesions, which are usually less than 1 cm. Most cases undergo spontaneous regression, leaving a small scar.

1. Morphology: Diffuse infiltration of large cohesive sheets of CD30+ tumor cells. The tumor cells are usually not epidermotropic.
2. Immunophenotype: Neoplastic cells are CD30+ (membrane and Golgi staining pattern), CD4+ or CD8+ (rare) with variable loss of CD2, CD5 and/or CD3, and expression of granzyme B, TIA1 and perforin. CD15, EMA and ALK are negative.
3. Cytogenetic and molecular studies: TCR gene rearrangement is positive in approximately 60% of the cases. t(2;5) translocation is absent.
4. Prognosis: The clinical outcome is better than that of peripheral T-cell lymphomas.

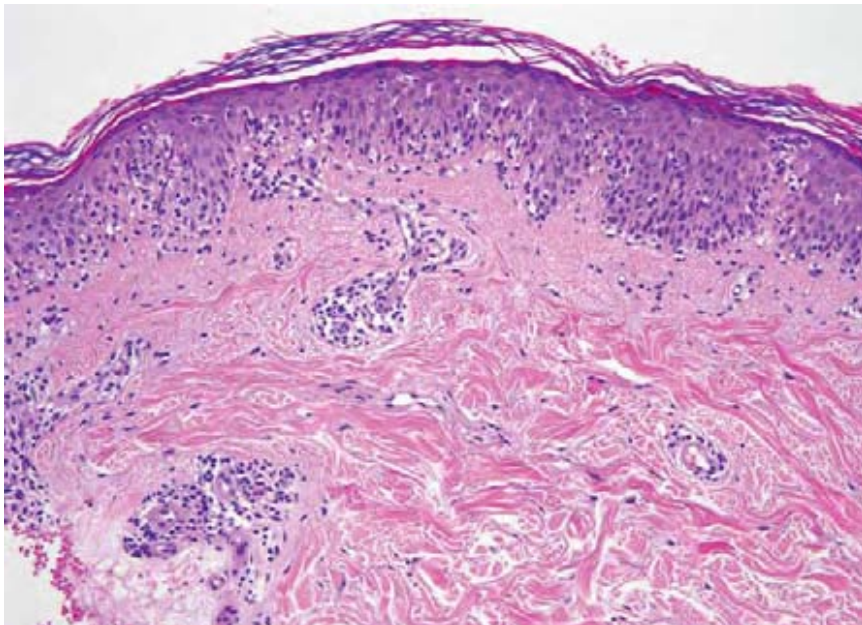
### ***Peripheral T-cell Lymphoma, Unspecified (PTCL, NOS)***

Peripheral T-cell lymphoma, unspecified is a heterogeneous group of nodal and extranodal mature T-cell lymphomas. The diagnosis is made when other specific entities have been excluded. Patients present with lymphadenopathy and frequently have “B” symptoms. Other extranodal sites such as liver,

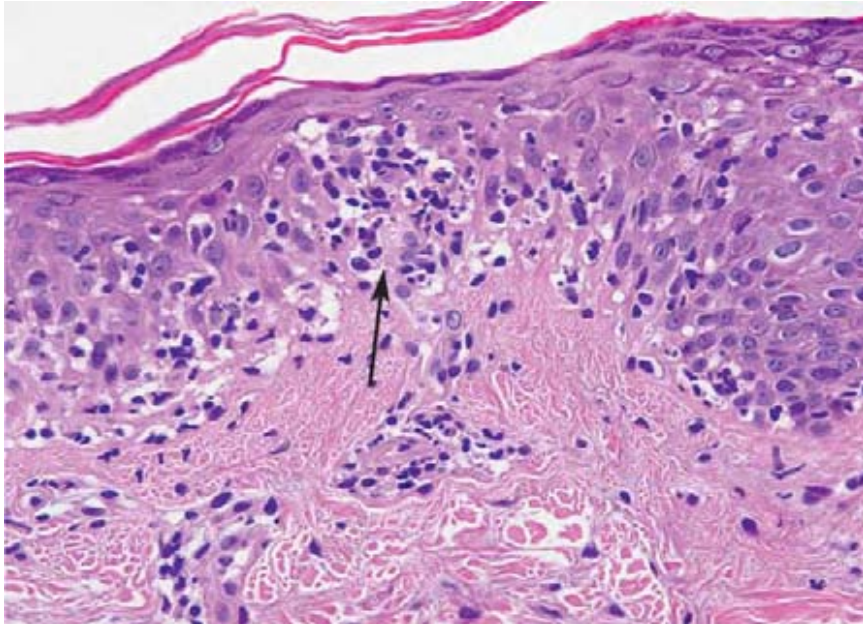




**Fig. 10-11A: Mycosis fungoides.** Skin punch biopsy showing the typical band-like lichenoid infiltrate at the dermal-epidermal junction (*Courtesy: Dr Garth Fraga, University of Kansas Medical Center, Kansas City, KS, USA*).



**Fig. 10-11B: Mycosis fungoides.** Skin punch biopsy showing the neoplastic T-cells infiltrating the epidermis (epidermotropism) (*Courtesy: Dr Garth Fraga, University of Kansas Medical Center, Kansas City, KS, USA*).



**Fig. 10-11C: Mycosis fungoides.** Skin punch biopsy showing the neoplastic T-cells forming small tumor cell aggregates within the epidermis (Pautrier microabscesses, arrow) (Courtesy: Dr Garth Fraga, University of Kansas Medical Center, Kansas City, KS, USA).

spleen, skin, lung, and gastrointestinal tract are commonly involved. The peripheral blood may be involved; however, a leukemic phase is uncommon.

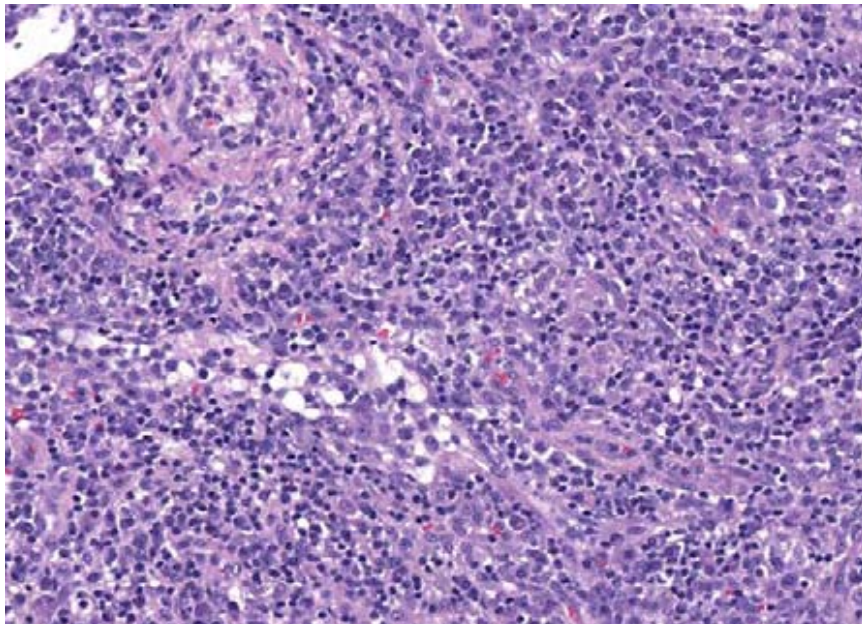
1. Morphology: Neoplastic cells vary in size. Eosinophils and epithelioid histiocytes are frequently present. Occasional Reed-Sternberg cells may be seen.
2. Immunophenotype: T-cell associated antigens CD2+, CD3+, CD5+/-, CD7+/-, CD4+ (more common than CD8+) with dropping of one or more pan T-cell markers.
3. Cytogenetic and molecular studies: TCR gene rearrangement is usually positive.
4. Prognosis: Clinical course is aggressive (Figs 10-12A to D).

### **Angioimmunoblastic T-cell Lymphoma**

Angioimmunoblastic T-cell lymphoma (AITL) is a peripheral T-cell lymphoma characterized by systemic disease, a polymorphous infiltration of lymph nodes, and a prominent proliferation of high endothelial venules and follicular dendritic cells.

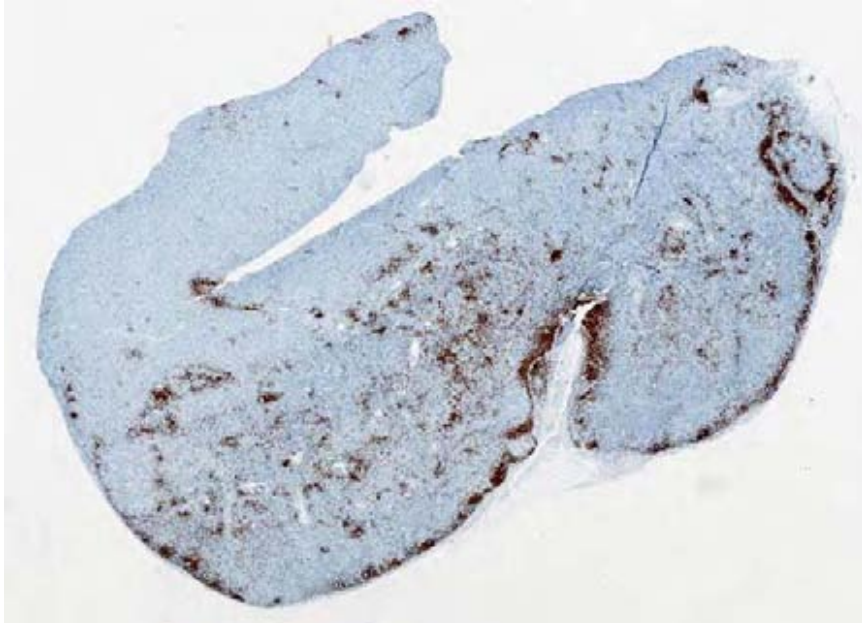


**Fig. 10-12A: Peripheral T-cell lymphoma, NOS.** Lymph node showing effacement of the normal architecture (Lymph node biopsy).



**Fig. 10-12B: Peripheral T-cell lymphoma, NOS.** At high magnification, the neoplastic T-cells are medium-sized with irregular, pleomorphic, hyperchromatic or vesicular nuclei and prominent nucleoli (Lymph node biopsy).





**Fig. 10-12C: Peripheral T-cell lymphoma, NOS.** Immunohistochemical stain for CD3 showing diffuse infiltration of T-cells (Lymph node biopsy).



**Fig. 10-12D: Peripheral T-cell lymphoma, NOS.** Immunohistochemical stain for CD5 showing markedly dropped off pan-T-cell marker.



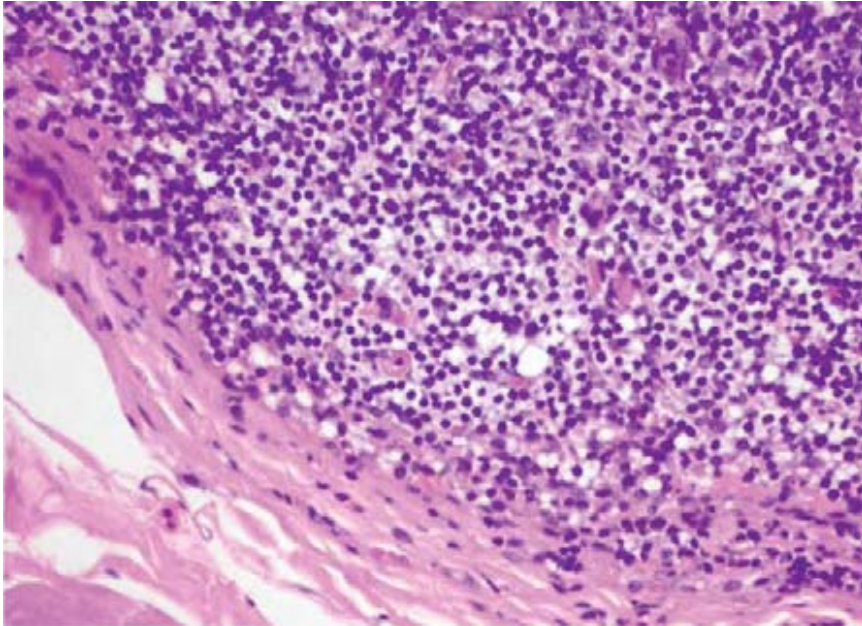
Patients usually present with generalized lymphadenopathy, fever, weight loss, skin rash, and polyclonal hypergammaglobulinemia. Many cases are associated with EBV infection and patients are at risk of developing B-cell lymphomas.

1. Morphology: The lymph node is partially effaced with sparing of the peripheral cortical sinuses. There is evidence of vascular proliferation by arborizing endothelial venules surrounded by proliferating follicular dendritic cells. The neoplastic cells have clear cytoplasm and form small aggregates commonly seen in the subcapsular region.
2. Immunophenotype: CD3+, CD4+ and CXCL13+, may show aberrant expression of CD10. Flow cytometry analysis is usually not helpful.
3. Cytogenetic and molecular studies: TCR gene rearrangement is usually positive; approximately 10% of the cases also show IgH gene rearrangement (due to EBV driven clonal B-cell proliferation) ( Figs 10-13A to G).

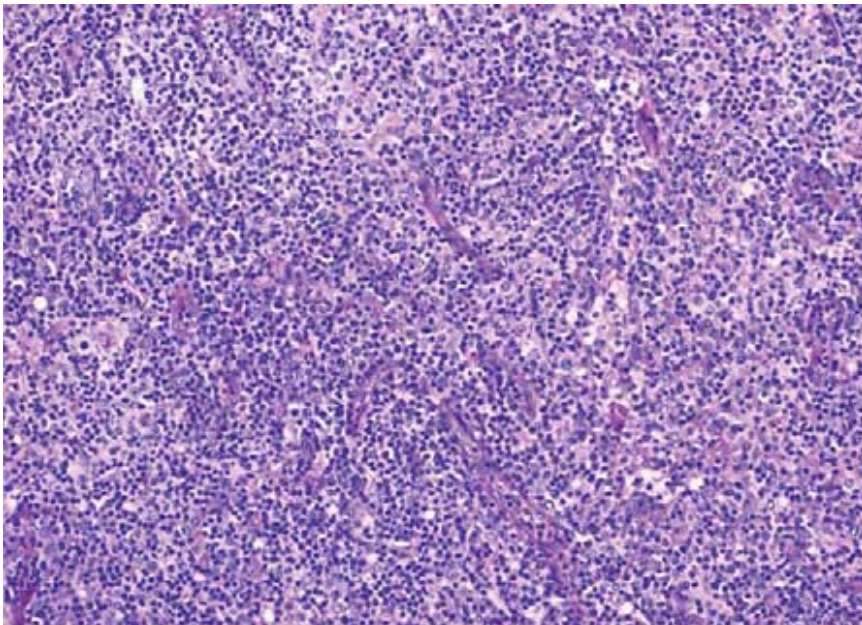
### ***Anaplastic Large Cell Lymphoma, ALK-positive***

Anaplastic large cell lymphoma (ALCL) , ALK+ is a T-cell lymphoma composed of large pleomorphic cells with abundant cytoplasm and eccentric horseshoe or kidney-shaped nuclei ( **Hallmark cells** ). Involvement of the ALK gene is characteristic. Extranodal site involvement includes skin, bone and soft tissue.

1. Morphology: A broad morphologic spectrum. Several morphologic patterns have been recognized and more than one pattern may present. All morphologic patterns contain “Hallmark” cells.
  - Common pattern (60% of cases): Prominent population of large CD30+ neoplastic cells with irregular kidney-shaped nuclei. Some neoplastic cells may resemble Reed-Sternberg cells.
  - Lymphohistiocytic pattern (10% of cases): CD30+ neoplastic cells admixed with large number of reactive histiocytes.
  - Small cell pattern (5-10% cases): Small to medium-sized neoplastic cells with irregular nuclei and pale rim of cytoplasm. “Hallmark” cells are present. This morphologic variant is often misdiagnosed as peripheral T-cell lymphoma, NOS. Flower-like cells may be seen on the smear if peripheral blood is involved.
  - Hodgkin-like pattern (3% cases): Morphological features mimic nodular sclerosing classic Hodgkin lymphoma.
2. Immunophenotype: Neoplastic cells are CD30+, EMA+, Clusterin+, ALK-1+, CD2+/CD5+/CD4+ (>70%), TIA1+, granzyme B+, perforin+, CD15-, CD3-(>75%), BCL2-, and CD8-.

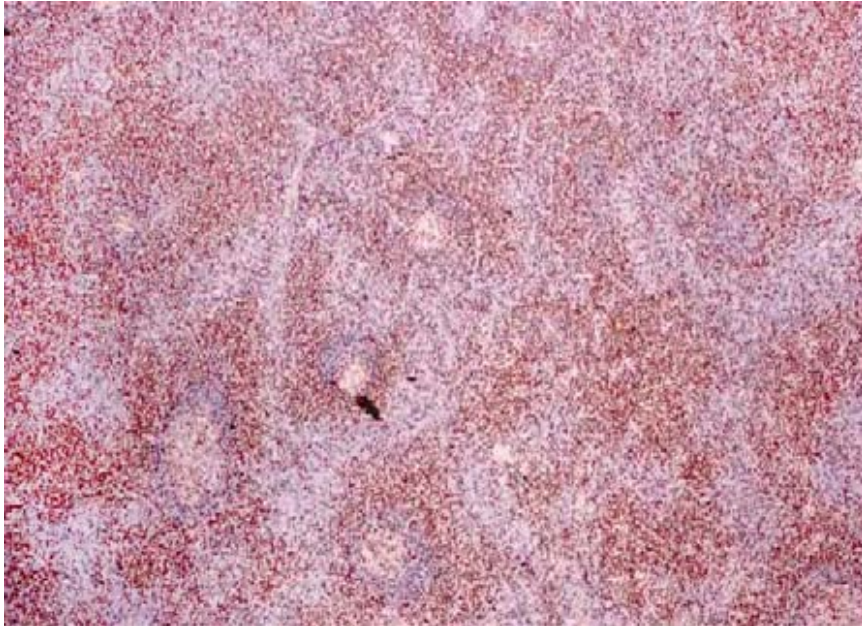


**Fig. 10-13A: Angioimmunoblastic T-cell lymphoma.** Clusters of neoplastic T-cells with clear cytoplasm are prominent underneath the capsule of the lymph node (Lymph node biopsy).

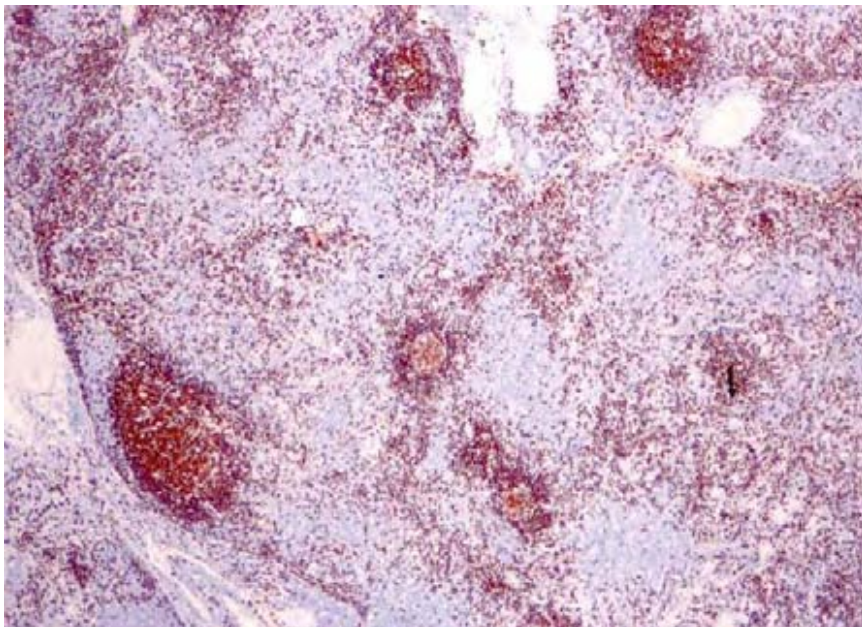


**Fig. 10-13B: Angioimmunoblastic T-cell lymphoma.** Lymph node biopsy showing prominent vascular proliferation and a pleomorphic lymphoid infiltrate (PAS stain).

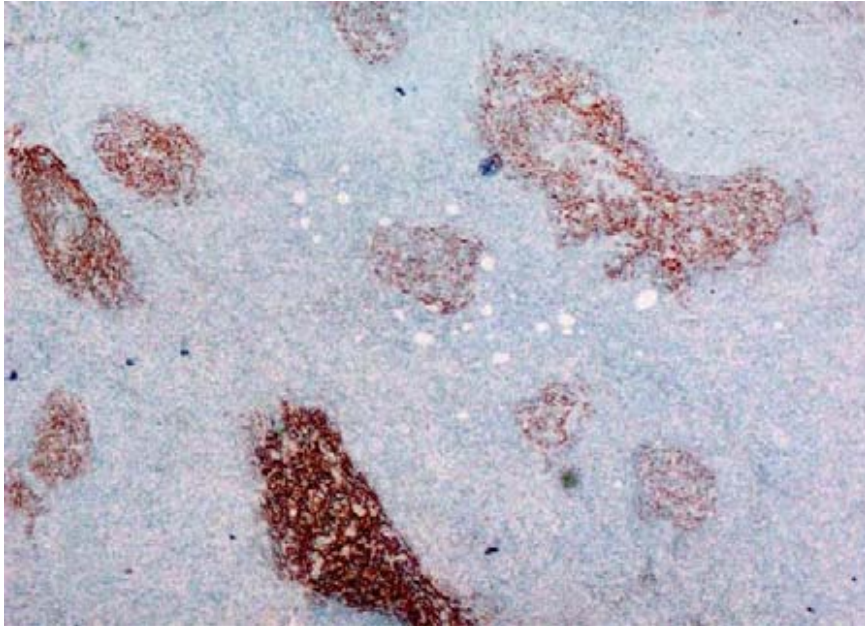




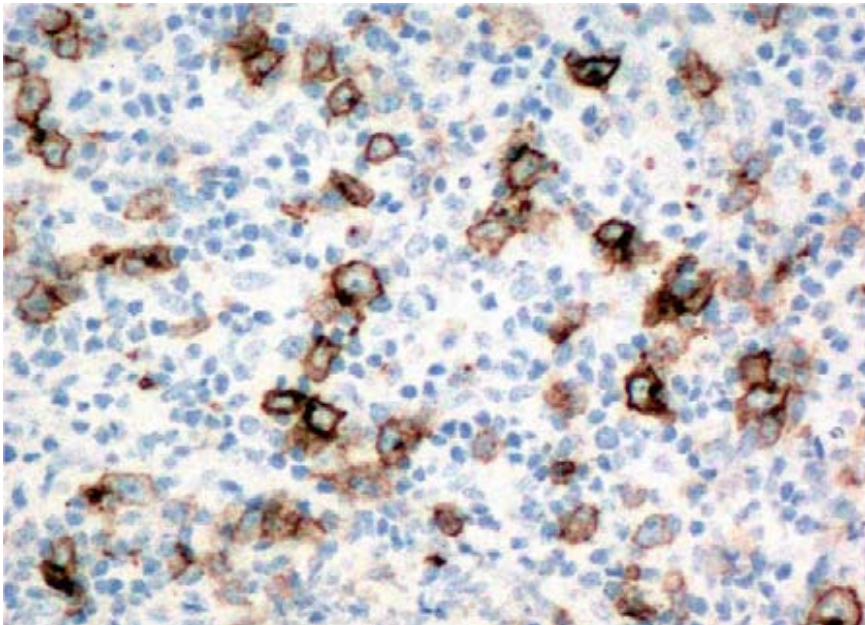
**Fig. 10-13C: Angioimmunoblastic T-cell lymphoma.** Immunohistochemical stain for CD3 showing diffuse infiltration of T-cells (Lymph node biopsy).



**Fig. 10-13D: Angioimmunoblastic T-cell lymphoma.** Immunohistochemical stain for CD20 shows residual follicles and scattered large immunoblasts between follicles, these immunoblasts are EBV positive (Lymph node biopsy).

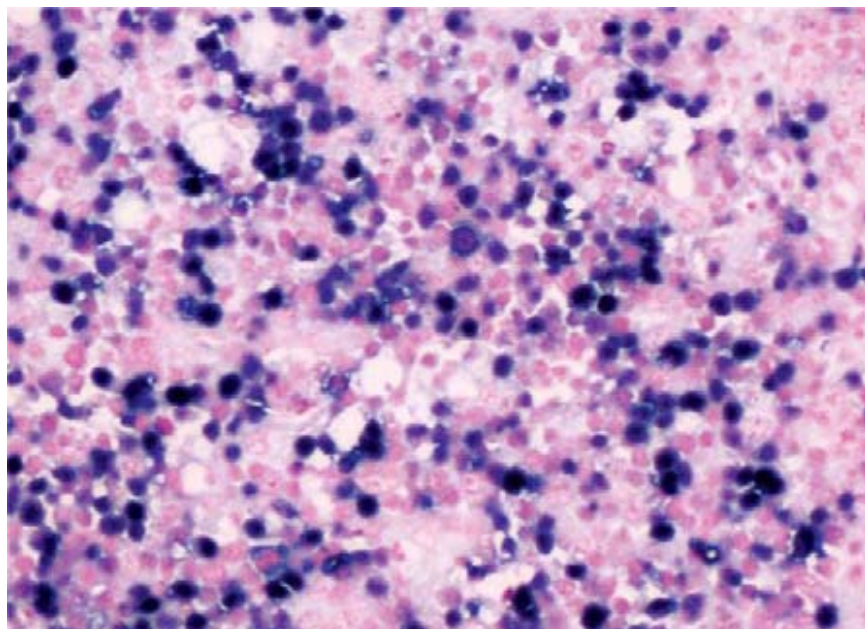


**Fig. 10-13E: Angioimmunoblastic T-cell lymphoma.** Immunohistochemical stain for CD21 shows residual follicles with a condensed network of follicular dendritic cells (Lymph node biopsy).



**Fig. 10-13F: Angioimmunoblastic T-cell lymphoma.** Immunohistochemical stain for CD30 highlights scattered large CD30 positive immunoblasts (Lymph node biopsy).





**Fig. 10-13G: Angioimmunoblastic T-cell lymphoma.** In situ hybridization for EBER shows scattered large EBV positive immunoblasts (Lymph node biopsy).

3. Cytogenetic and molecular studies: See Table 10-5.
4. Prognosis: ALK positive patients have an overall better survival rate compared to ALK negative patients (Figs 10-14A to D).

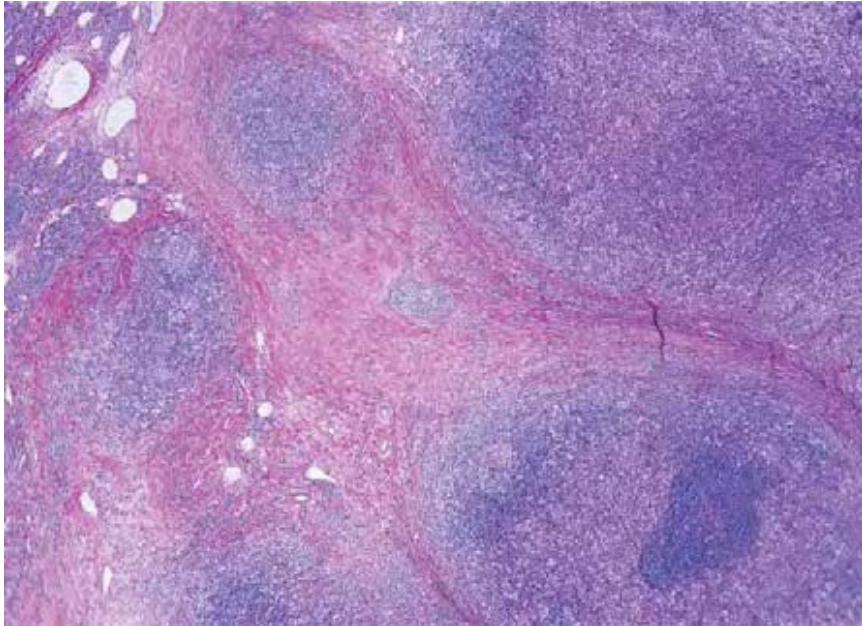
**TABLE  
10-5**

**Molecular and cytogenetic features of ALCL**

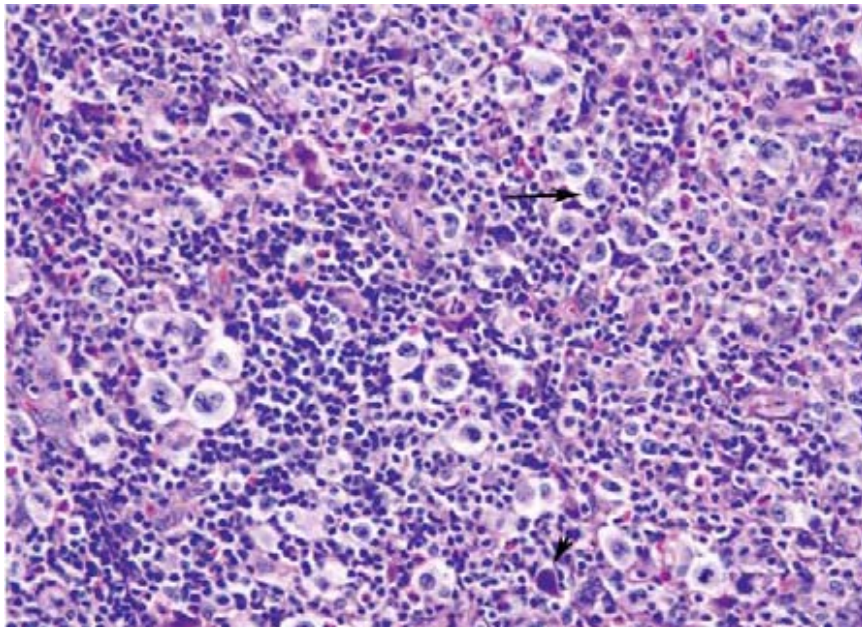
Translocation	Fusion protein	ALK stain pattern	Percentage(%)
t(2;5)(p23;q35)	ALK-NPM	Nuclear and cytoplasmic	84
t(1;2)(q25;p23)	TMP2-ALK	Cytoplasmic	13
Inv(2)(p23q35)	ALK-AT1C	Cytoplasmic	1
t(2;X)		Membrane	<1
t(2;3)		Cytoplasmic	<1
t(2;17)		Cytoplasmic	<1
t(2;19)		Cytoplasmic	<1
t(2;22)		Cytoplasmic	<1

ALK gene is located on chromosome 2.

Note: ALK expression may also be present in some non-hematopoietic neoplasms such as rhabdomyosarcoma and inflammatory myofibroblastic tumors.

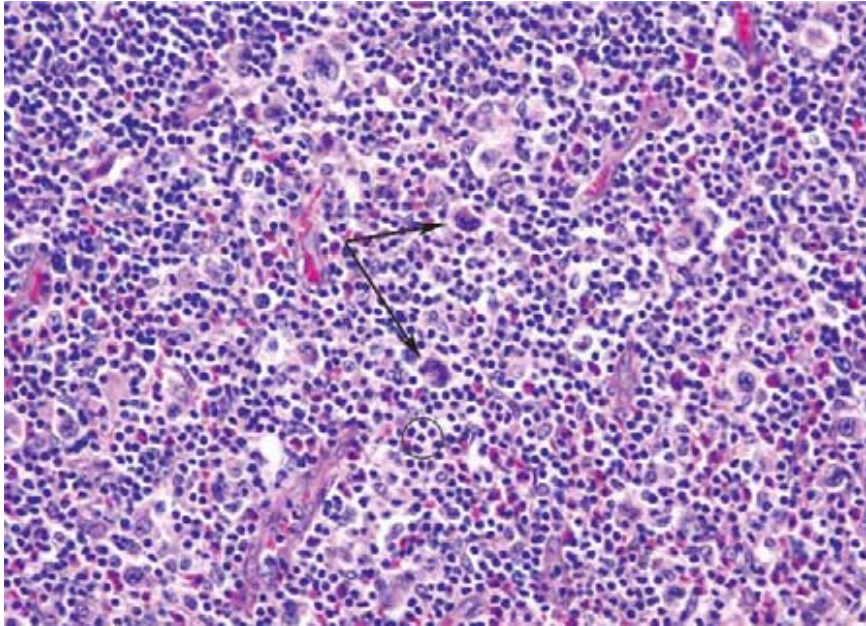


**Fig. 10-14A: Anaplastic large cell lymphoma (ALCL), Hodgkin-like pattern.** Fibrous collagen bands divide the lymph node into nodules (Lymph node biopsy).

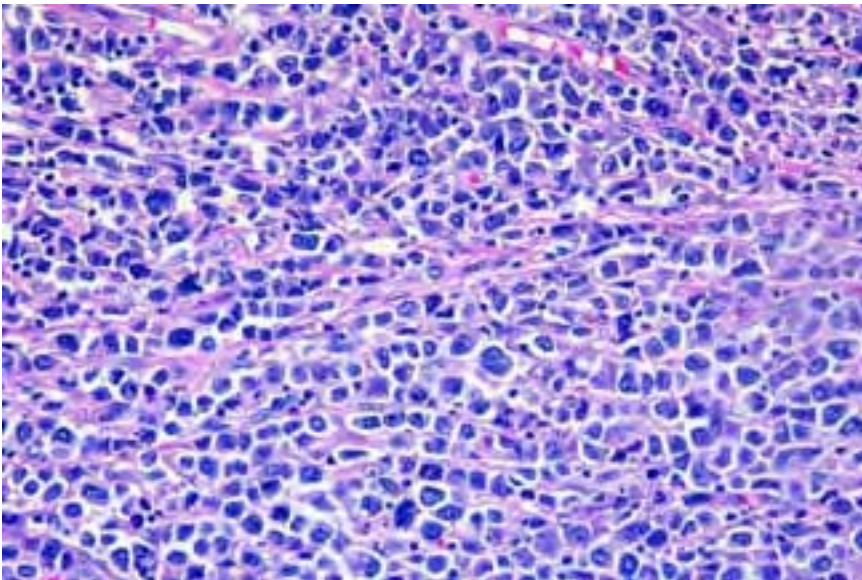


**Fig. 10-14B: Anaplastic large cell lymphoma (ALCL), Hodgkin-like pattern.** At high magnification, lacunar cells, Reed-Sternberg like cells (arrow), and mummified cells (arrowhead) are present in a background of small lymphocytes and eosinophils. These large cells are positive for CD30 and a cytoplasmic and nuclear staining pattern of ALK-1 (not shown) (Lymph node biopsy).





**Fig. 10-14C: Anaplastic large cell lymphoma (ALCL), Hodgkin-like pattern.** "Horseshoe" like hallmark cells are present in all ALCL variants (arrows). The background small lymphocytes (circle) have a rim of clear cytoplasm. Lymphocytes with a rim of clear cytoplasm are commonly observed in peripheral T-cell or marginal zone B-cell lymphomas (Lymph node biopsy).



**Fig. 10-14D: Anaplastic large cell lymphoma (ALCL).** Lymph node showing anaplastic malignant lymphocytes with multiple nucleoli. "Horseshoe" like hallmark cells are present. ALK-1 is negative (Lymph node biopsy).

***Anaplastic Large Cell Lymphoma (ALCL), ALK-negative  
(provisional entity in WHO 2008)***

ALK negative anaplastic large cell lymphoma is a CD30+ T-cell lymphoma that morphologically resembles ALK positive anaplastic large cell lymphoma but the ALK stain is negative. This entity must be distinguished from primary cutaneous anaplastic large cell lymphoma, subtypes of CD30+ T- and B-cell lymphomas with anaplastic features, and classical Hodgkin lymphoma. ALK negative anaplastic large cell lymphomas have been reported in breast implant patient. Most patients present with persistent seromas, capsular contractures, or peri-implant masses. Anaplastic large cells present in the effusion fluid (seroma) surrounding the implant, in the fibrous capsule, or within a peri-implant mass. Typically, there was no invasion beyond the fibrous capsule into the breast parenchyma. Adjacent lymph nodes may also be involved in a cohesive and intrasinusoidal pattern (Figs 10-15A to E).

***Plasma Cell Neoplasms***

Plasma cell neoplasms arise from terminally differentiated B-cells that produce monoclonal immunoglobulin protein (Table 10-8).

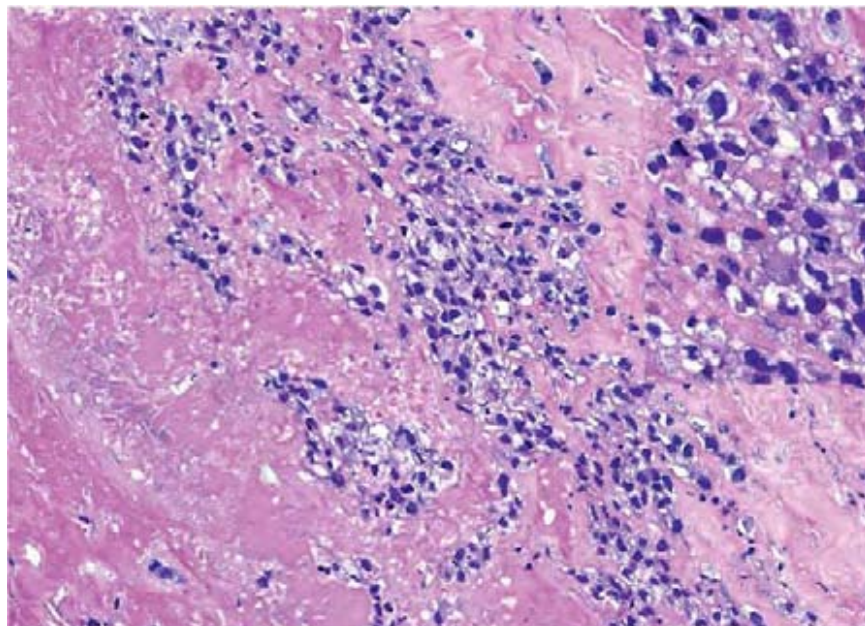
Neoplastic plasma cell proliferation (myeloma) in the bone marrow results in paraprotein formation, bone destruction and bone pain (often in the lower back). Myeloma may involve the skeleton at multiple sites (multiple myeloma). Solitary myeloma (plasmacytoma) may also arise in the tissue other than bone marrow (extramedullary plasmacytoma). Plasmacytoma may cause spinal cord compression. The light chain component of the immunoglobulin often leads to kidney failure and may be deposited in tissues as amyloid resulting in worsening kidney failure and systemic symptoms. Myeloma patients are prone to infections, especially by encapsulated organisms. Prognosis has been correlated with serum levels of  $\beta_2$ -microglobulin and cytogenetic abnormalities (Tables 10-6 and 10-7).

The International Staging System for myeloma relies on two factors:  $\beta_2$ -microglobulin and albumin (Table 10-7).

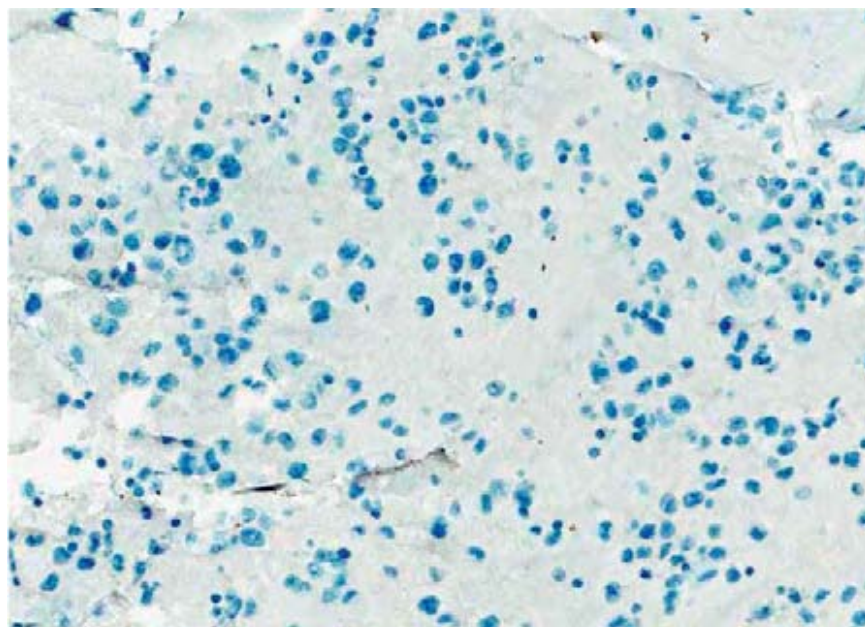
***Classification of Plasma Cell Neoplasms***

1. Monoclonal gammopathy of undetermined significance (MGUS)
2. Plasma cell myeloma
  - Variants:
    1. Asymptomatic (smoldering) myeloma

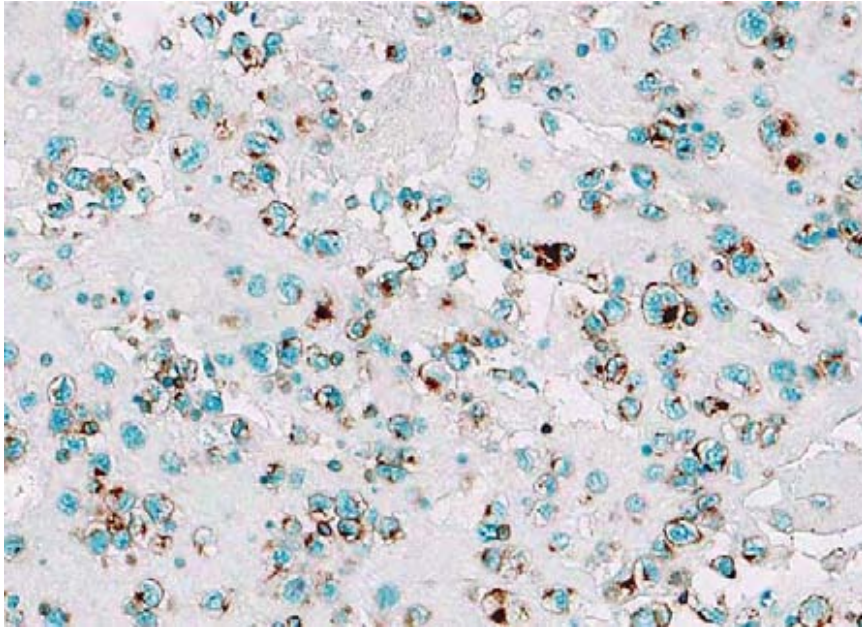




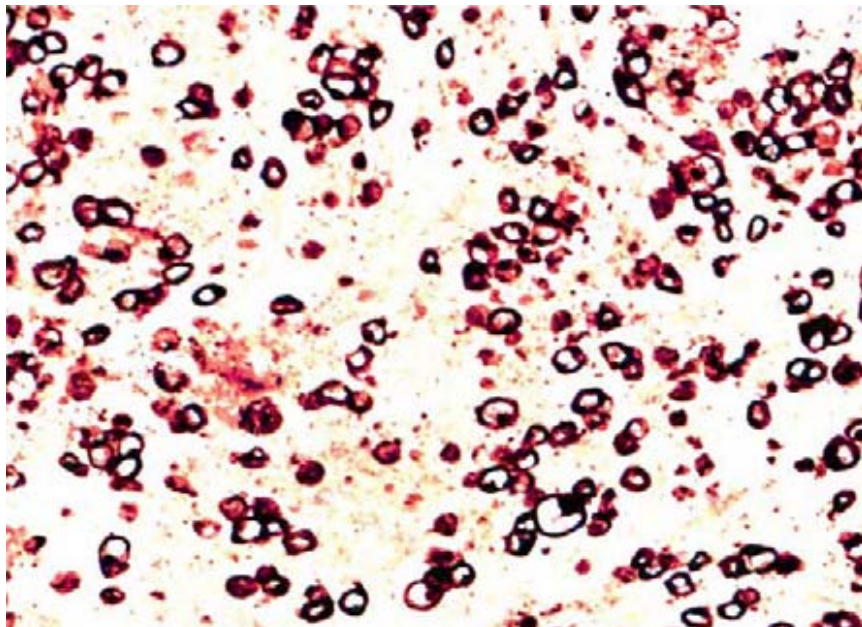
**Fig. 10-15A: Anaplastic large cell lymphoma, T-cell type.** The section is from a fibrotic capsule surrounding a silicon breast implant that shows clusters of large atypical cells infiltrating the capsule (inset).



**Fig. 10-15B: Anaplastic large cell lymphoma, T-cell type.** Immunohistochemical stain for ALK-1 is negative (Cell block from breast fluid accumulation).

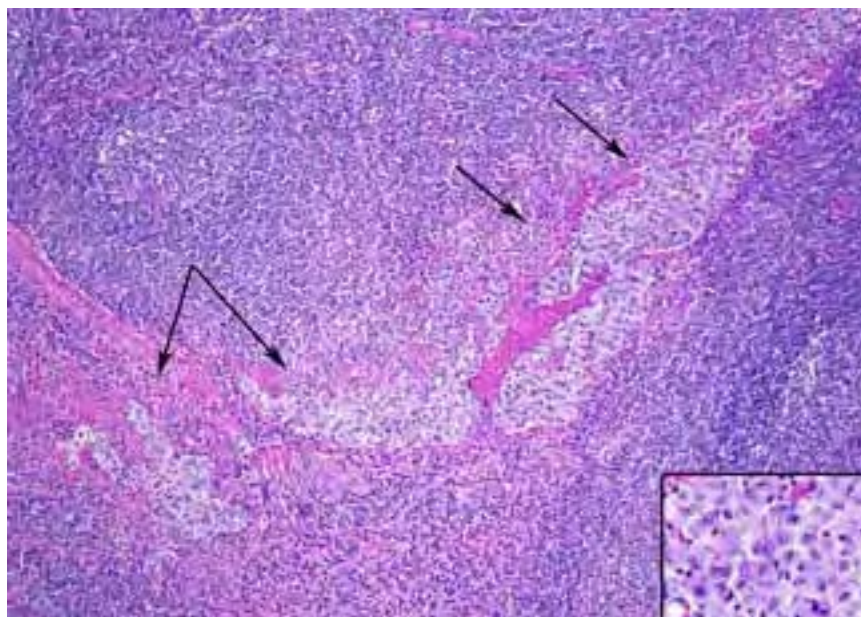


**Fig. 10-15C: Anaplastic large cell lymphoma, T-cell type.** Immunohistochemical stain for CD4 shows positive large atypical cells (Cell block from breast fluid accumulation).



**Fig. 10-15D: Anaplastic large cell lymphoma, T-cell type.** Immunohistochemical stain for CD30 highlights large atypical cells (membrane and Golgi-staining patterns) (Cell block from breast fluid accumulation).





**Fig 10-15E: Anaplastic large cell lymphoma, T-cell type.** Axillary lymph node of the same patient shows anaplastic large cell (inset) in a cohesive and intrasinusoidal growth pattern (Lymph node).

**TABLE  
10-6**

**Cytogenetic abnormalities and prognosis of plasma cell myeloma**

**Unfavorable:**

Deletion 13, or aneuploidy by karyotype analysis  
t(4;14) or t(14;16) or t(14;20) by FISH  
Deletion 17p13 by FISH  
Hypodiploidy

**Favorable:**

Hyperdiploid  
t(11;14) or t(6;14) by FISH

**TABLE  
10-7**

**International staging system for plasma cell myeloma**

Stage	Criteria	Median survival
I	Serum $\beta_2$ -microglobulin <3.5 mg/L, albumin >3.5 g/dl	62 months (~ 5 years)
II	Not stage I or III*	44 months (~ 3.6 years)
III	Serum $\beta_2$ -microglobulin >5.5 mg/L	29 months (~ 2.5 years)

\* Serum  $\beta_2$ -microglobulin <3.5 mg/L but albumin <3.5 g/dl, or Serum  $\beta_2$ -microglobulin 3.5 to <5.5 mg/L regardless albumin level.

**TABLE  
10-8****Monoclonal immunoglobulin and plasma cell disorders**

Disease	Immunoglobulin	Light chain restriction
Myeloma	IgG (55%) IgA (22%) Light chain only (18%) IgD (2%)	κ common κ common κ common λ common
Heavy chain disease	γ (25%) α (75%) μ (5%)	
MGUS	IgM (100%)	
Primary amyloidosis		λ common

2. Non-secretory myeloma
3. Plasma cell leukemia
3. Plasmacytoma
  1. Solitary plasmacytoma of bone
  2. Extramedullary plasmacytoma
4. Immunoglobulin deposition disease
  1. Primary amyloidosis
  2. Systemic light and heavy chain deposition disease
5. Osteosclerotic myeloma (POEMS syndrome).

### Monoclonal Gammopathy of Undetermined Significance

Monoclonal gammopathy of undetermined significance (MGUS) is an elevation of a clonal immunoglobulin in the serum that is generally present at <3 g/dL. The paraprotein in MGUS is IgG (70%), IgM (15%), IgA (12%) or biclonal (3%). MGUS is considered a pre-neoplastic condition, as it may evolve into plasma cell myeloma, amyloidosis, Waldenström macroglobulinemia, or other lymphoproliferative disorders. MGUS occurs in about 1% in people 50 years and older and in up to 10% of 75 years and older. The risk of progression is about 1% per year and indefinite. The risk of progression is also related to the level of M-protein and the type of immunoglobulin (IgM and IgA have greater risk).

Cytogenetic and molecular studies show approximately 40% of patients to have hyperdiploidy, 50% to have a 14q32 translocation. Other abnormalities are also present.



***Diagnostic Criteria for MGUS (WHO 2008)***

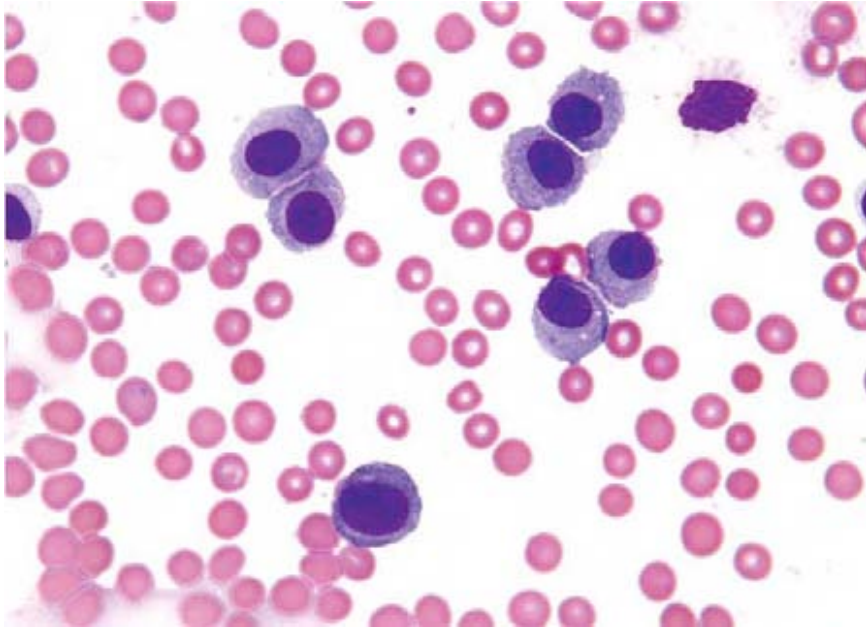
1. Serum M-protein <30 g/L.
2. <10% monoclonal plasma cells in the bone marrow.
3. No lytic bone lesions.
4. No myeloma-related end organ damage ( **CRAB**: hyperCalcemia, **R**enal insufficiency, **A**nemia, **B**one lesions).
5. No evidence of other B-cell proliferative disorders.

**Diagnostic Criteria for Plasma Cell Myeloma (WHO 2008)**

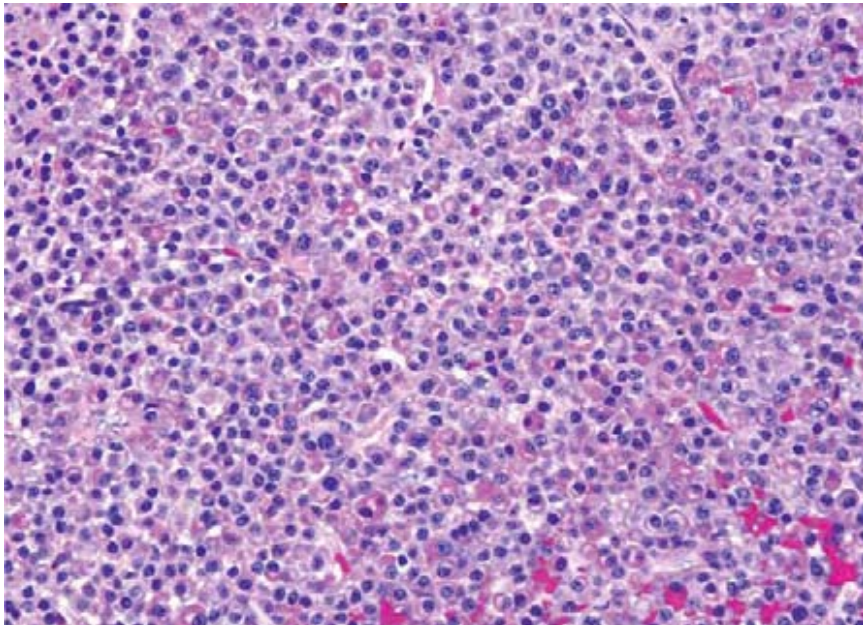
1. Symptomatic plasma cell myeloma
  - a. M-protein in serum or urine
  - b. Bone marrow clonal plasma cells or plasmacytoma
  - c. Related end-organ damage (**CRAB**: hyper Calcemia, **R**enal insufficiency, **A**nemia, **B**one lesions).
2. Asymptomatic (smoldering) myeloma
  - a. Serum M-protein at myeloma level (>30 g/L) and/or ≥10% monoclonal plasma cells in the bone marrow.
  - b. No myeloma-related end-organ damage ( **CRAB**: hy perCalcemia, **R**enal insufficiency, **A**nemia, **B**one lesions) or myeloma-related symptoms.

**Variants of Plasma Cell Myeloma**

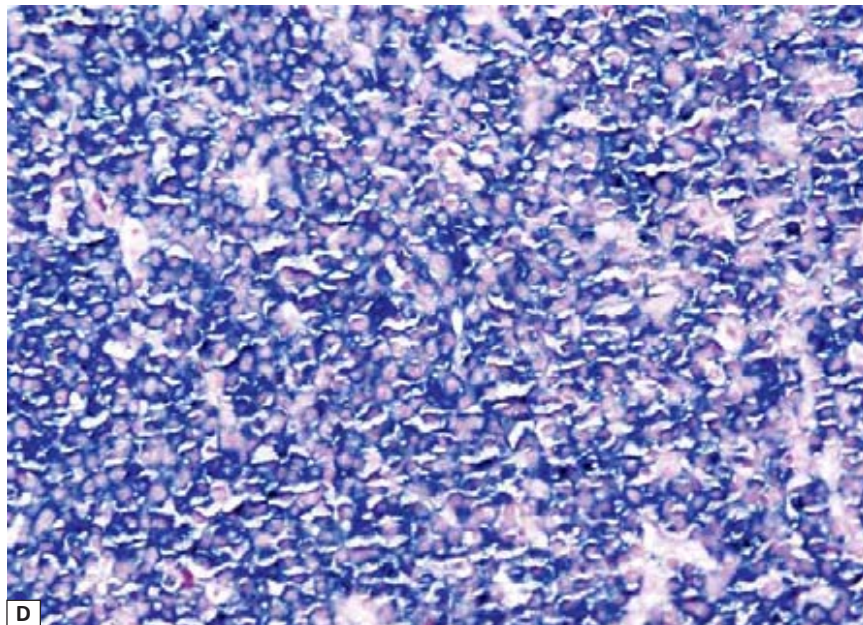
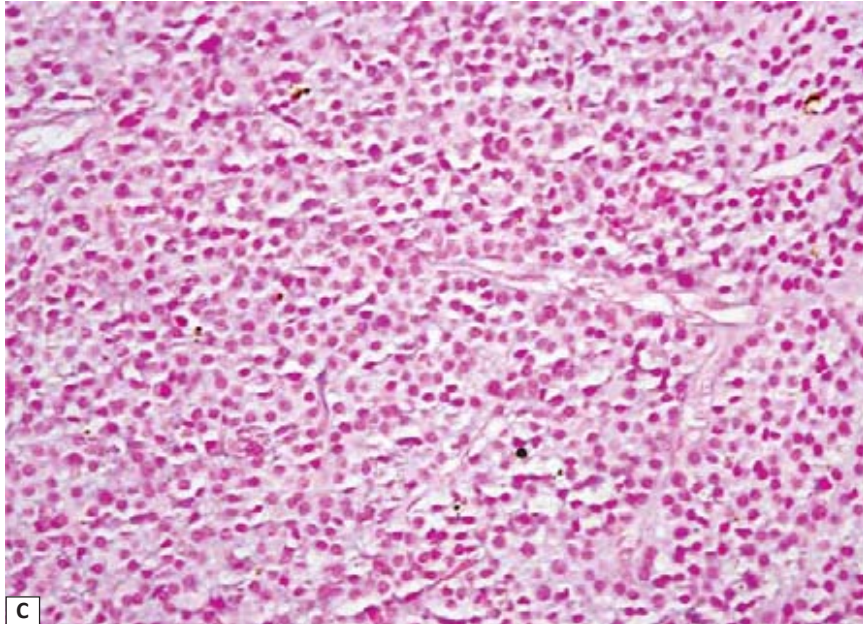
1. Non-secretory myeloma: No detectable monoclonal protein in the urine or serum.
2. Asymptomatic (smoldering) myeloma: Patients are asymptomatic and have a stable clinical course.
3. Plasma cell leukemia: Primary (no previous myeloma diagnosis) or secondary (previous myeloma diagnosis). Diagnostic criteria is based on the peripheral blood containing greater than **20%** plasma cells or an absolute plasma cell count greater than **2000/μl** ( $>2 \times 10^9/L$ ) (Fig. 10-16A).
4. Extramedullary plasmacytoma  
Common locations for these tumors are in the head and neck region (nasal cavity, nasopharynx and sinuses) (Figs 10-16B to D).
5. Heavy chain disease  
Rare, characterized by secretion of heavy chains without associated light chains. Immunophenotypic studies and/or serum/urine immunofixation shows monotypic gamma heavy chains.



**Fig. 10-16A: Plasma cell leukemia.** Peripheral blood smear showing markedly increased plasma cells. Diagnostic criteria: >20% plasma cells or an absolute plasma cells >2000/ $\mu$ l in the peripheral blood.



**Fig. 10-16B: Plasmacytoma.** Plasma cells have eosinophilic cytoplasm which contain immunoglobulins.



**Figs 10-16C and D: Plasmacytoma.** In situ hybridization for Kappa light chain (negative, C) and lambda light chain (positive, D) demonstrate the malignant monoclonal population of plasma cells.



There are three types:

1.  $\gamma$  (25% of the cases) with features of lymphoplasmacytic lymphoma.
2.  $\alpha$  (75% of the cases) a variant of extranodal marginal zone lymphoma.
3.  $\mu$  (less than 5% of the cases) with features similar to chronic lymphocytic leukemia.

## Amyloidosis and Systemic Light and Heavy Chain Deposition Disease

1. Amyloidosis: Most of patients have M-protein. The diagnosis is confirmed by tissue biopsy with Congo red stain showing apple green birefringence under polarized light or electron microscopy showing a typical fibrillar structure (Figs 10-16E to H).
2. Systemic light and heavy chain deposition disease is characterized by visceral and soft tissue amorphous material deposition and accumulation. The light or heavy chain deposition leads to compromised organ function or organ failure (Fig. 10-16I).

## Osteosclerotic Myeloma (POEMS Syndrome)

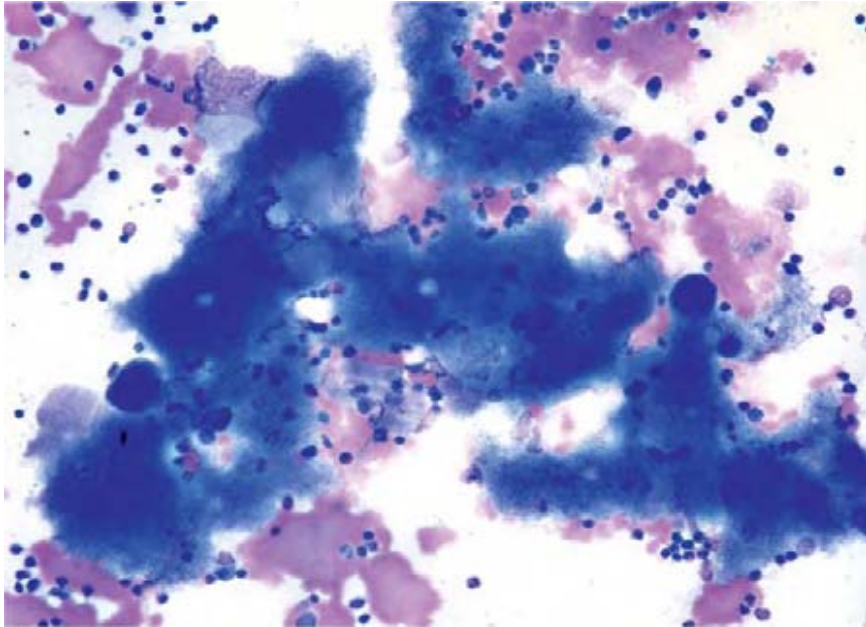
The features of POEMS syndrome are **P**olyneuropathy (most common feature), **O**rganomegaly (~50% of cases), **E**ndocrinopathy (~60% of cases), **M**onoclonal gammopathy or **M**ultiple myeloma or **M** spike (~85% of cases), and **S**kin changes (~60% of cases).

Patients usually have a severe, progressive sensorimotor polyneuropathy associated with sclerotic bone lesions from myeloma. Unlike typical myeloma, hepatomegaly and lymphadenopathy occur in about 60% of patients, and splenomegaly is seen in approximately 30% of the cases. A biopsy from the lymph nodes frequently resembles **multicentric plasma cell variant of Castleman's disease**.

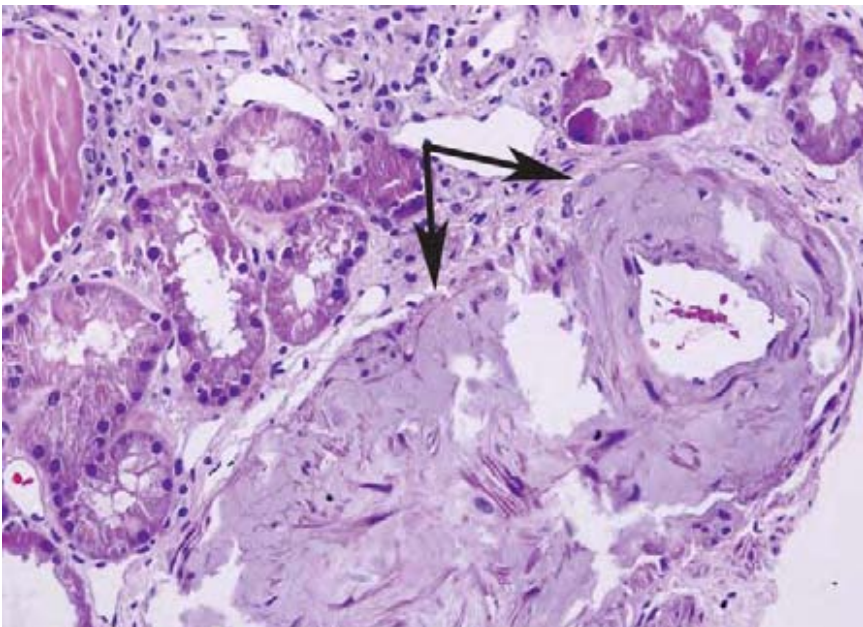
## Cryoglobulinemia

Cryoglobulins are immunoglobulins that reversibly precipitate from serum and plasma at cool temperatures. Cryoglobulinemia is commonly associated with an underlying disease process.

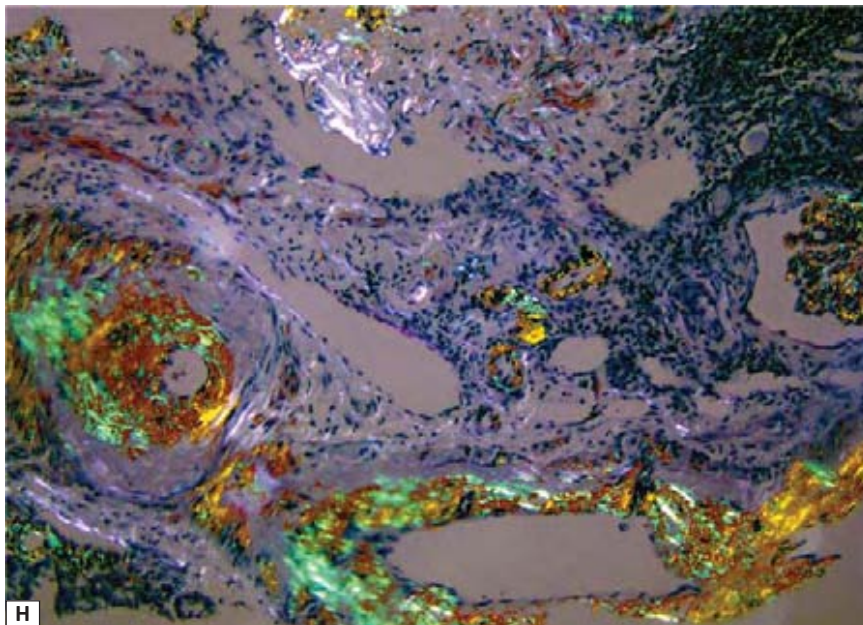
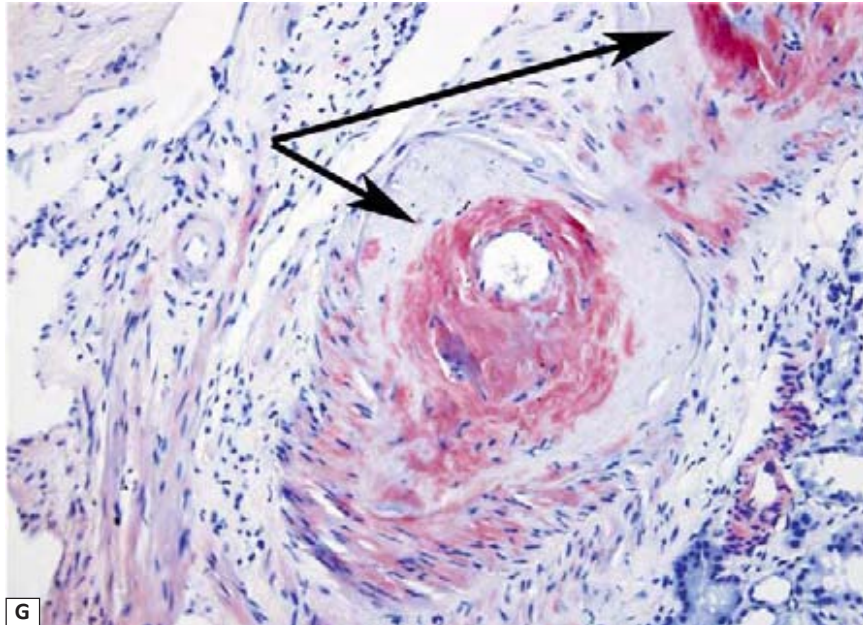




**Fig. 10-16E: Amyloidosis.** The bone marrow aspirate showed diffuse deposits of amyloid stained by Giemsa. The amyloid appear as amorphous, nebulous navy blue material (Bone marrow aspirate).

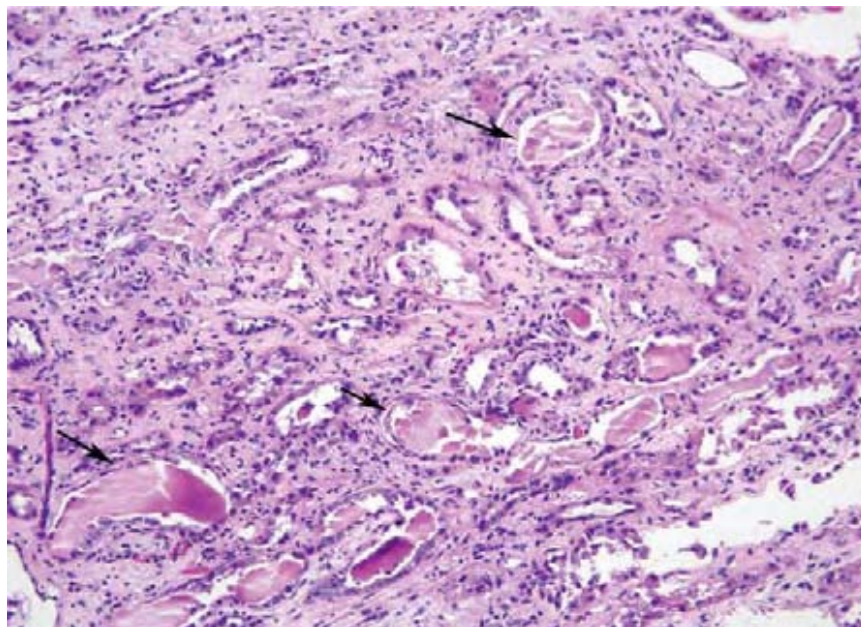


**Fig. 10-16F: Amyloidosis.** Kidney core biopsy showing amorphous eosinophilic deposits in the vessel wall.



**Figs 10-16G and H: Amyloidosis.** Kidney core biopsy with Congo red stain for amyloid. The section shows the light microscopy finding of salmon pink-colored amyloid deposition in a vessel wall (G). The same kidney core biopsy with Congo red stain for amyloid now under polarized light; the amyloid deposition is apple-green and birefringent (H).





**Fig. 10-16I: Myeloma light chain cast nephropathy.** Kidney core biopsy from a multiple myeloma patient showing acute tubular injury with amorphous eosinophilic material (light chain deposition) in the lumen of tubules (arrows). Light chain cast nephropathy occurs in the presence of excess free light chains in the plasma and urine. The intratubular cast formation, direct tubular toxicity, and light chain precipitation in the tubules lead to obstruction, tubular injury and renal failure.

### ***Three Types of Cryoglobulinemia***

1. Type I cryoglobulinemia

The cryoglobulin is a monoclonal immunoglobulin (usually IgM, less commonly IgG or IgA). High circulating levels of immunoglobulin may result in significant hyperviscosity. This type is typically associated with an underlying lymphoproliferative disorder such as chronic lymphocytic leukemia, Waldenström macroglobulinemia, multiple myeloma, monoclonal gammopathy, or other B-cell lymphomas.

2. Type II cryoglobulinemia

In this type, the cryoglobulin is a mixture of monoclonal immunoglobulin (usually IgM) and polyclonal immunoglobulin (usually IgG).

3. Type III cryoglobulinemia

The cryoglobulin is composed entirely of polyclonal immunoglobulin (usually IgG), without a monoclonal component.

Types II and III are mixed cryoglobulinemia and are more common than type I. Type II and type III are associated with systemic lupus erythematosus, Sjögren syndrome, and hepatitis C viral infection.

### Laboratory Evaluation

Blood specimen requirements include drawing into a prewarmed non-anticoagulated tube and transportation in a 37°C container to prevent cryoglobulin precipitation. Serum will be separated (at 37°C) and incubated at 4°C for 3-7 days. If precipitation is present at 4°C but dissolves after warming the specimen, the test is positive.

### Histiocytic and Dendritic Cell Neoplasms

Macrophages, histiocytes, Langerhans cells, interdigitating dendritic cells (IDC) and follicular dendritic cells (FDC) are all antigen-presenting cells. The immunophenotypic comparison of antigen-presenting cells and their neoplasms is listed in Table 10-9.

**TABLE  
10-9**

Comparison of antigen presenting cells

	Macrophage	Langerhans cell	IDC	FDC
CD1a	+	+	—	—
CD3	—	—	—	—
CD4	+	+	—	—
CD20	—	—	—	—
CD21	—	—	—	++
CD35	—	—	—	++
CD68	+	—	—	—
CD163	+	—	—	—
Lysozyme	+	—	—	—
S-100	+/-	+	+	—

IDC = interdigitating dendritic cells

FDC = follicular dendritic cells

### Langerhans Cell Histiocytosis (LCH)

Langerhans cell histiocytosis is a clonal proliferation of Langerhans cells that usually involves bone (skull, ribs, pelvis, and femur). Langerhans cell histiocytosis is more common in the Caucasian population and rarely occurs in African Americans. It is most often seen in older children. The male to female ratio is 3.7 to 1. Langerhans cell histiocytosis may be associated with other hematopoietic neoplasms (Hodgkin lymphoma, Non-Hodgkin lymphoma or ALL) and smoking.

Langerhans cell histiocytosis may present in a variety of ways including a solitary lesion (eosinophilic granuloma), multiple lesions (Hand-Schüller-



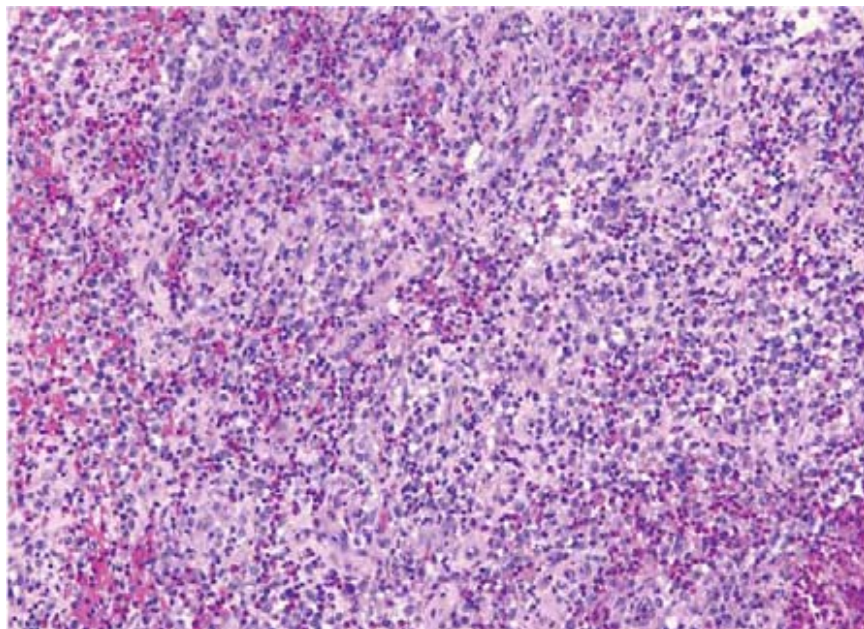
Christian disease) or disseminated/visceral involvement (Letterer-Siwe disease).

The neoplastic Langerhans cells usually form a sheet with scattered eosinophils. These Langerhans cells have nuclear grooves and are CD1a and S-100 positive. On electron microscope examination, the Langerhans cells contain **Birbeck granules** in the cytoplasm (Figs 10-17A to E).

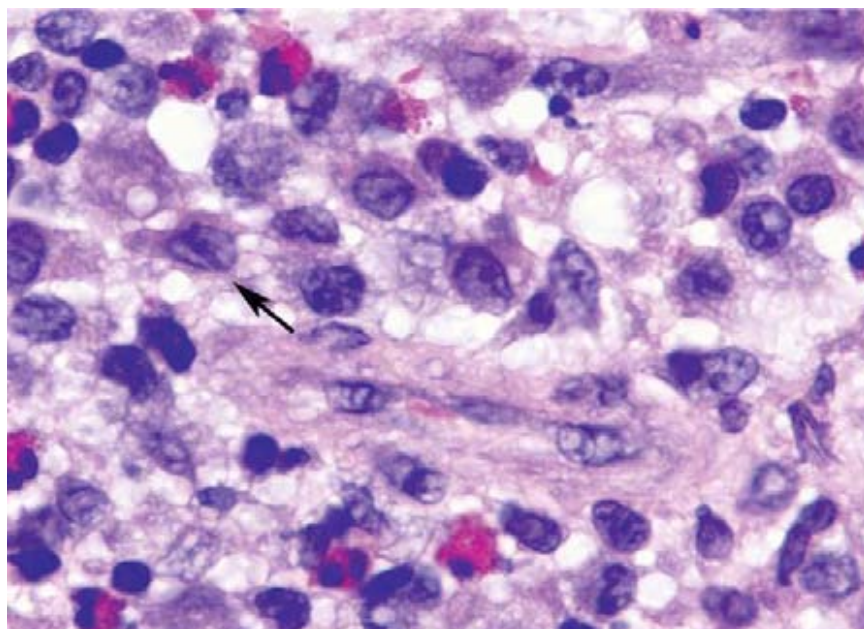
### **Histiocytic Sarcoma**

Histiocytic sarcoma is rare. Follicular lymphoma may transform to histiocytic or dendritic sarcoma.

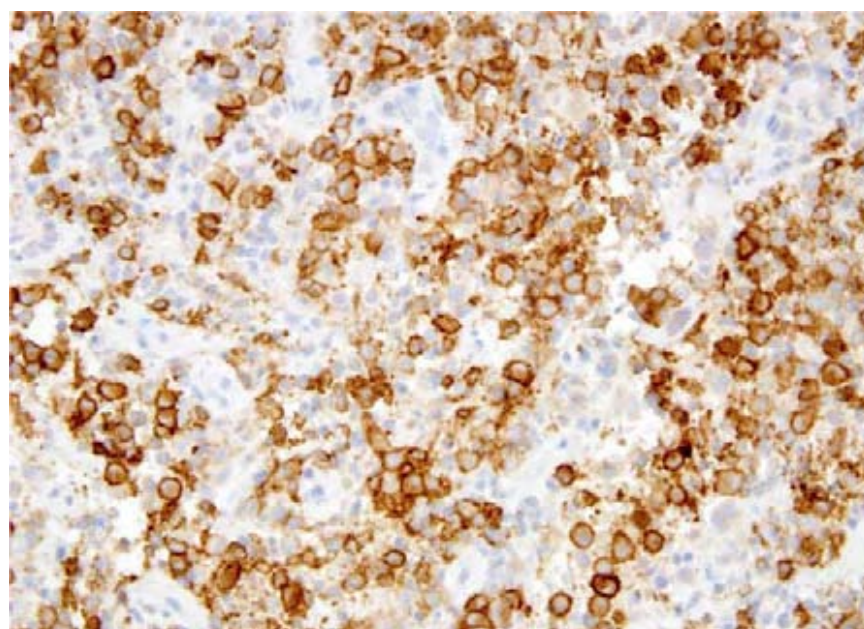
The tumor cells usually express histiocytic markers CD68, CD163 and lysozyme. No Birbeck granules are identified in the cytoplasm by electron microscope examination. Histiocytic sarcoma may be confused with diffuse large B-cell lymphoma or syncytial variant of Hodgkin lymphoma partially treated with steroids before biopsy. Immunohistochemical studies are important to confirm the diagnosis (Fig. 10-18).



**Fig. 10-17A: Langerhans cell histiocytosis.** Bone marrow section showing sheets of Langerhans cells, histiocytes and scattered eosinophils.

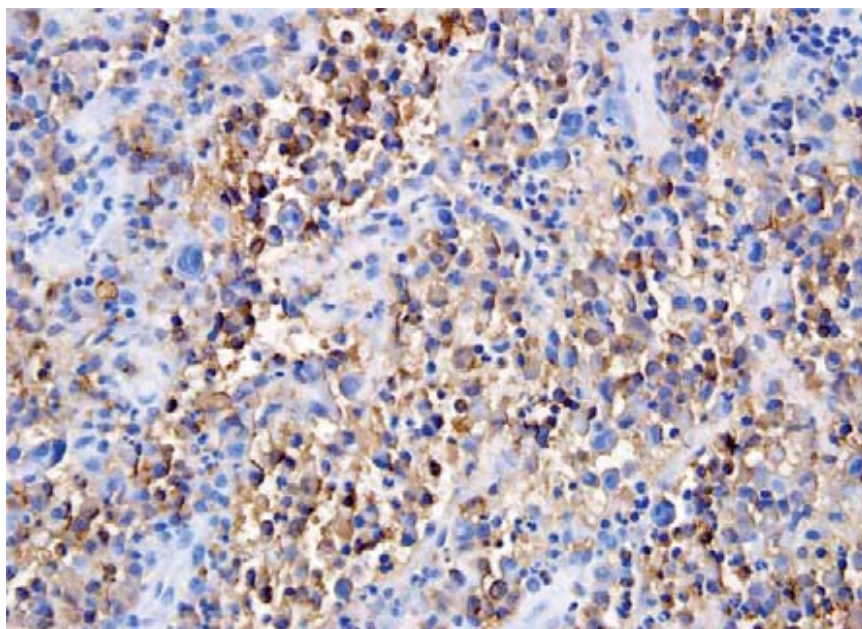


**Fig. 10-17B: Langerhans cell histiocytosis.** Bone marrow section (high magnification) showing the nuclear grooves (arrow) characteristic of Langerhans cells.



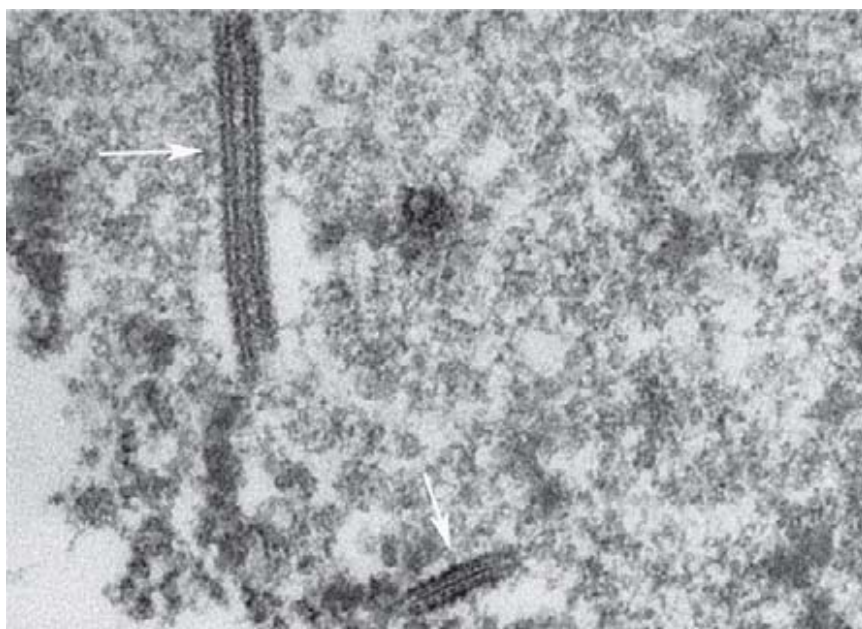
**Fig. 10-17C**



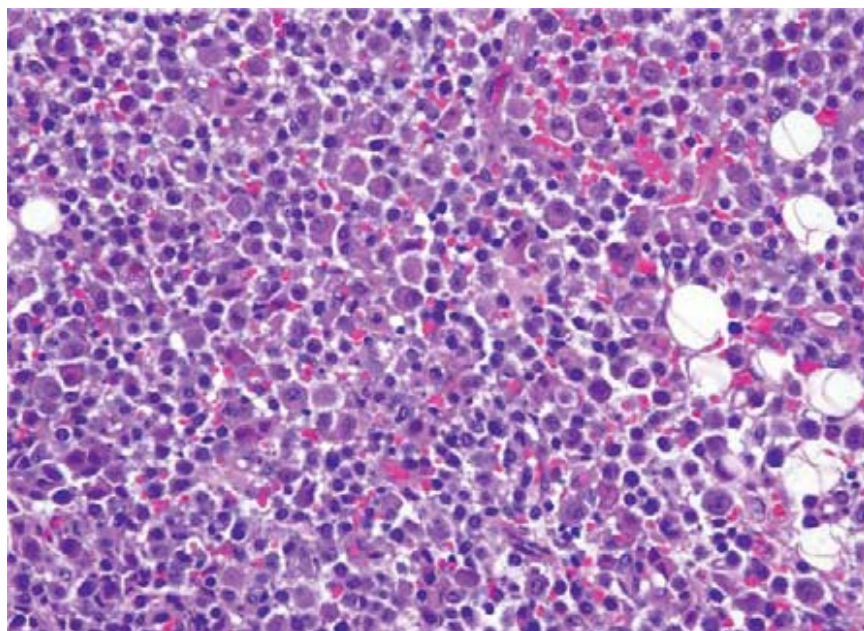


**Fig. 10-17D**

**Figs 10-17C and D: Langerhans cell histiocytosis.** Immunohistochemical stains for CD1a (C) and S100 (D) highlighting the Langerhans cells (Bone marrow section).



**Fig. 10-17E: Langerhans cell histiocytosis.** Electron micrograph showing the Langerhans cell to contain typical Birbeck granules in the cytoplasm (arrows). The Birbeck granules have a characteristic racquet shape and are specific to Langerhans cells (Bone marrow section).



**Fig. 10-18: Histiocytic sarcoma.** Liver section showing large tumor cells with abundant cytoplasm. May be confused with diffuse large B-cell lymphoma. Immunohistochemical stain for histiocytic marker CD68 is positive (not shown).

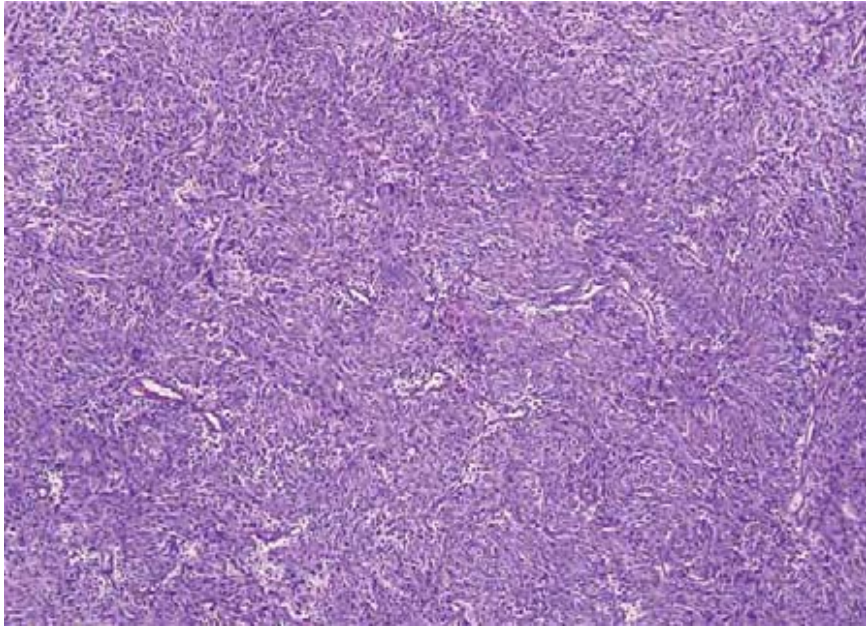
### Follicular Dendritic Cell Sarcoma

Follicular dendritic cell tumor is rare. 10-20% of the cases are associated with hyaline-vascular type Castleman's disease. Immunohistochemical studies for follicular dendritic markers (CD21, CD23, CD35, and clusterin) are important to confirm the diagnosis (Figs 10-19A and B).

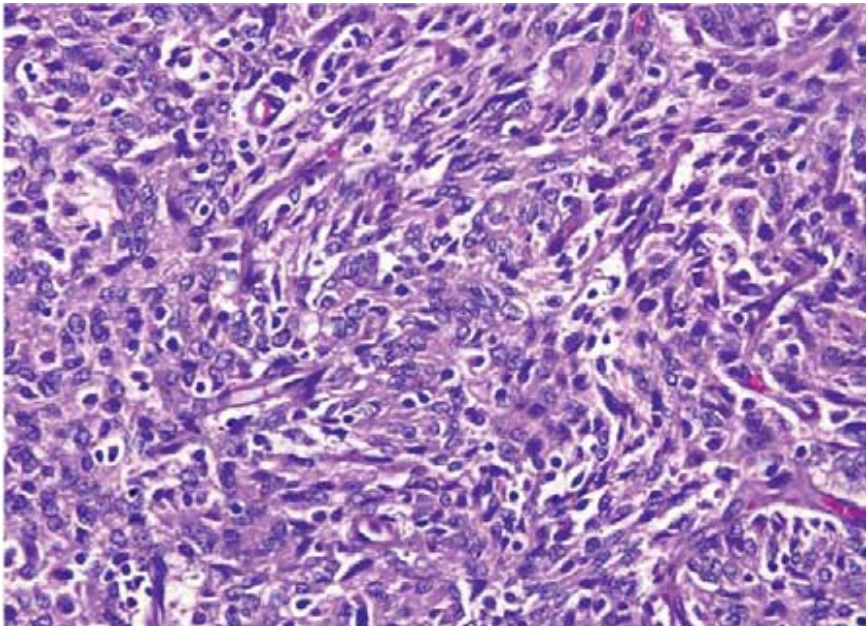
### Interdigitating Dendritic Cell Sarcoma

Interdigitating dendritic cell tumor is very rare. Immunohistochemical studies ruling out other sarcomas and histiocytic/dendritic neoplasms are important to confirm the diagnosis (Figs 10-20A and B).



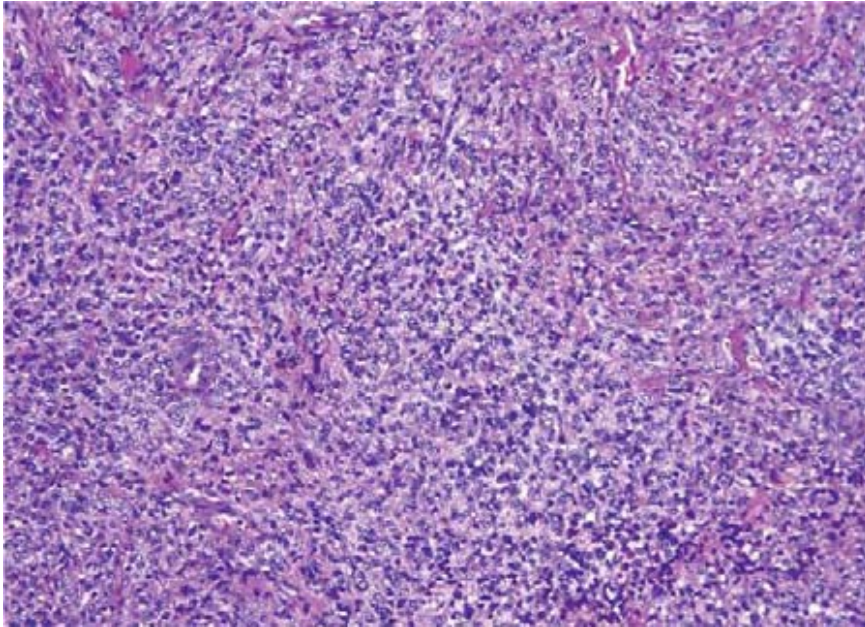


**Fig. 10-19A: Follicular dendritic cell sarcoma.** Lymph node biopsy showing a proliferation of spindle cells producing a whorled appearance. May be associated with Castleman's disease.

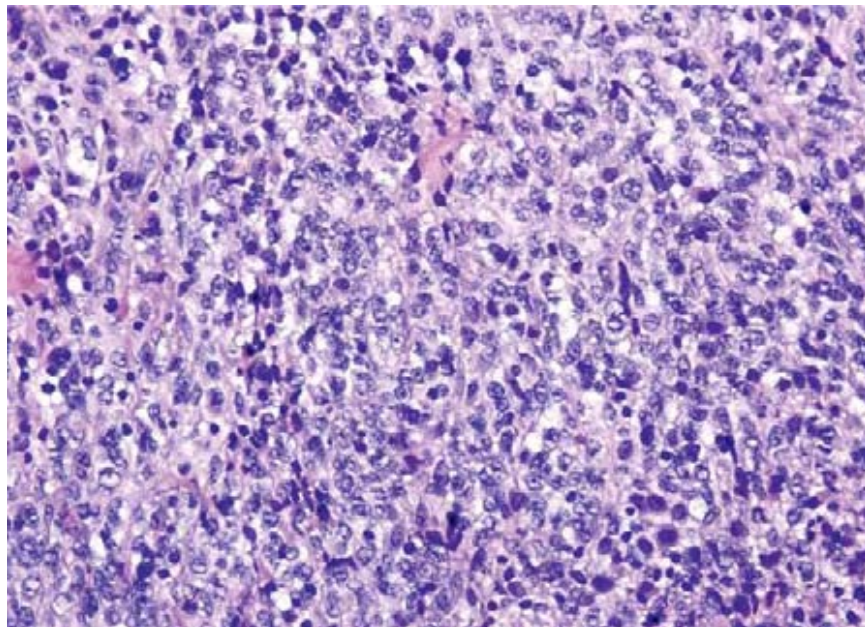


**Fig. 10-19B: Follicular dendritic cell sarcoma.** Lymph node biopsy showing ovoid tumor cells with indistinct cytoplasmic outlines.





**Fig. 10-20A: Interdigitating dendritic cell sarcoma.** Lymph node biopsy showing the total effacement of the normal lymph node.



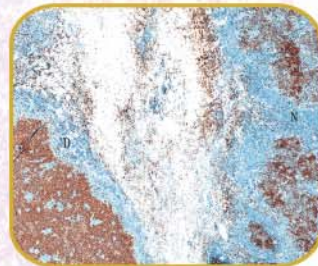
**Fig. 10-20B: Interdigitating dendritic cell sarcoma.** Lymph node biopsy showing tumor cell pleomorphism (round, oval or spindle-shape). May be confused with follicular dendritic cell sarcoma. Immunohistochemical stains are important to confirm the diagnosis.



CHAPTER

11

# Hodgkin Lymphoma



Hodgkin lymphoma (HL) is a group of lymphomas characterized by Reed-Sternberg cells or lymphocyte predominant cells (LP cells) in an appropriate reactive cellular background. These malignant cells are derived from B-lymphocytes.

Hodgkin lymphoma is divided into two types: nodular lymphocyte predominant Hodgkin lymphoma and classical Hodgkin lymphoma. Based on morphology, classical Hodgkin lymphoma is divided into four histological subtypes. Classical Hodgkin lymphoma is characterized by multinucleated Reed-Sternberg cells residing in a background of mixed non-neoplastic reactive inflammatory cells. Reed-Sternberg cells are derived from mature B-cells at the germinal center stage of differentiation, and have monoclonal immunoglobulin gene rearrangements. However, Reed-Sternberg cells have lost much of the B-cell specific markers. Classical Hodgkin lymphoma includes four subtypes and accounts for 95% of Hodgkin lymphoma. Nodular lymphocyte predominant Hodgkin lymphoma accounts for about 5% of the cases. Morphologic and immunophenotypic features distinguish classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma.

Hodgkin lymphoma has a tendency to arise within a single lymph node area and spreads in an orderly fashion to contiguous lymph nodes. Hodgkin lymphoma is classified into four stages, I to IV (Table 11-1).

Radiotherapy and chemotherapy have resulted in high cure rates in a majority of those patients who are younger than 65 years old.

### Classification of Hodgkin Lymphoma (WHO 2008)

1. Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)
2. Classical Hodgkin lymphoma (CHL)
  1. Nodular sclerosis (NSCHL)
  2. Lymphocyte-rich (LRCHL)
  3. Mixed cellularity (MCCHL)
  4. Lymphocyte-depleted (LDCHL).

### Nodular Lymphocyte Predominant Hodgkin Lymphoma

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) accounts for 5% of all Hodgkin lymphomas. Unlike classical Hodgkin lymphoma, nodular lymphocyte predominant Hodgkin lymphoma has a **single peak** in the fourth decade of life. Patients usually present with localized disease, and a history of previous biopsy usually shows reactive follicular hyperplasia or



**TABLE  
11-1****Ann Arbor staging of Hodgkin lymphoma**

Stage	Findings
I	Involvement of single lymph node region or lymphoid structure (spleen, thymus, Waldeyer ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the number of sites should be indicated with suffix)
III	Involvement of lymph node regions or structures on both side of diaphragm III <sub>1</sub> : with or without splenic, hilar, celiac or portal nodes III <sub>2</sub> : with paraaortic, iliac, or mesenteric nodes
IV	Involvement of extranodal site(s) beyond those designated E

E: involvement of a single extranodal site, contiguous or proximal to a known nodal site of disease.

Other terms have been used in staging:

A: no symptoms

B: fever, drenching sweats, weight loss, pruritus (B symptoms)

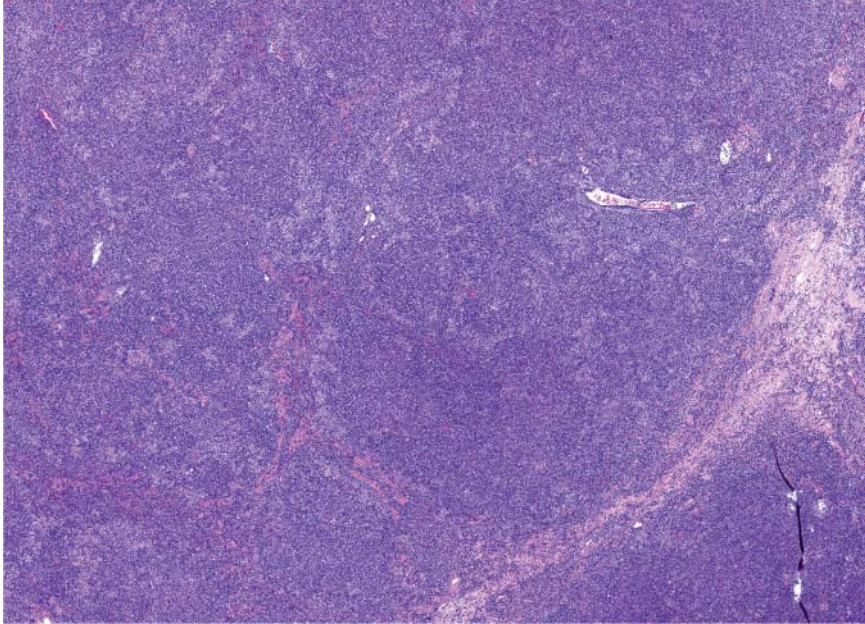
X: bulky disease

**progressive transformed germinal centers (PTGC).** Involvement of mediastinum is uncommon.

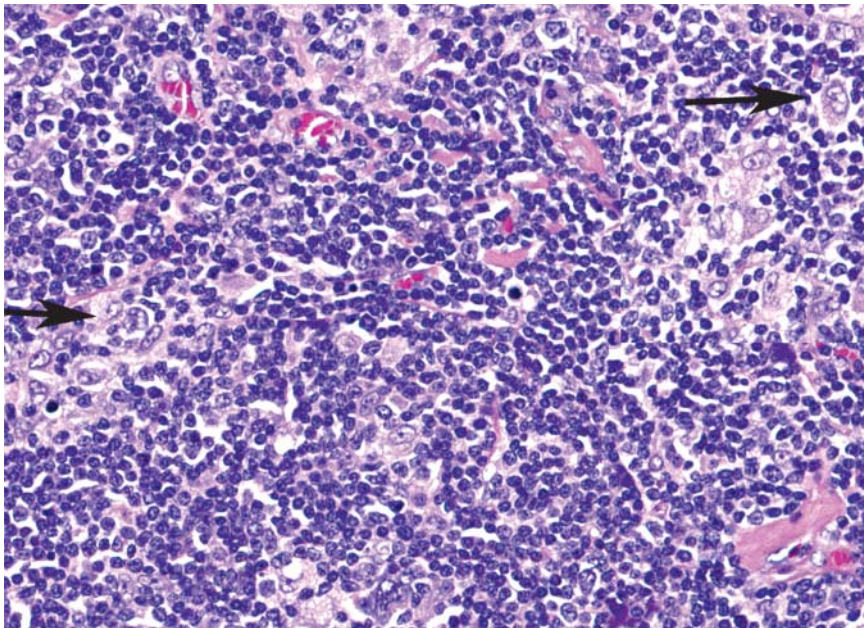
1. Morphology shows a nodular pattern at low power microscopic examination with scattered lymphocyte predominant (LP) cells (AKA “Popcorn cells”). LP cells are multilobated nuclei with small nucleoli.
2. Immunophenotype of LP cells: CD20+, CD45+, CD79a+, CD15-, and CD30-/+.
4. Differential diagnosis: T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) (*See* Table 10-4).
5. Prognosis: Approximately 3-5% of the cases progress to diffuse large B-cell lymphoma. If localized, the prognosis is good (Figs 11-1A to G).

## Classical Hodgkin Lymphoma

Classical Hodgkin lymphoma (CHL) represents 20-30% of lymphomas in USA and Europe, and 10% of lymphomas in Asia. Classical Hodgkin lymphoma has a **bimodal** age specific incidence at 15-45 years old and 60 years old. The clinical presentation is usually painless cervical lymphadenopathy and 25% of Hodgkin lymphoma patients have “B” symptoms. Nodular sclerosis subtype may have mediastinal involvement. Mixed cellularity subtype is more commonly associated with abdominal and splenic involvement. Bone marrow involvement and primary extranodal disease is unusual. There is a

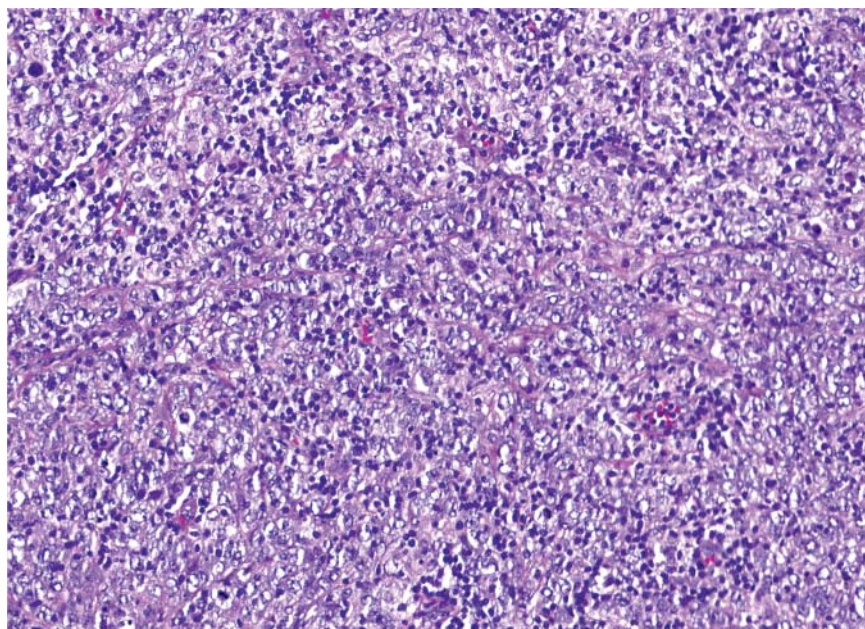


**Fig. 11-1A: Nodular lymphocyte predominant Hodgkin lymphoma.** Lymph node biopsy showing numerous vague nodules, tightly packed without mantle zones. Band-like sclerosis/fibrosis may present.

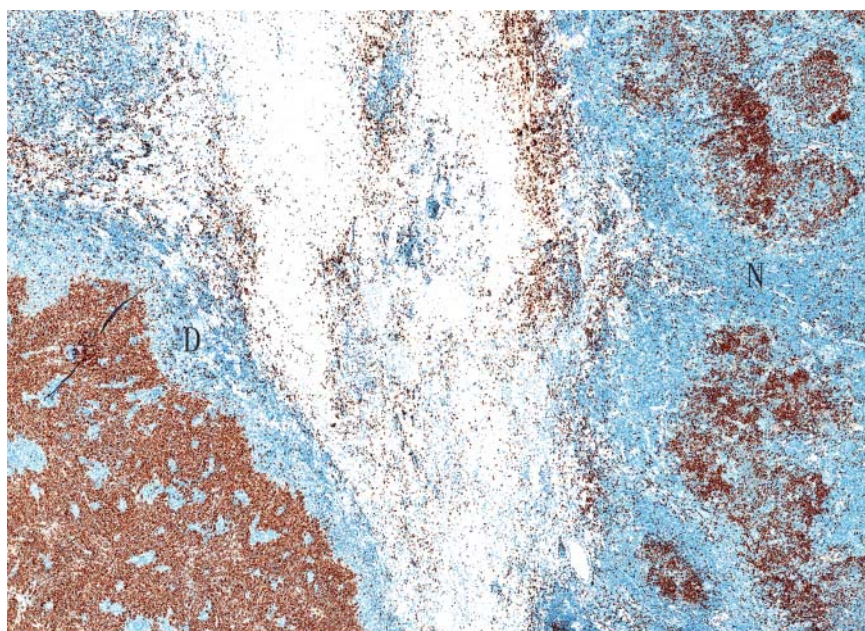


**Fig. 11-1B: Nodular lymphocyte predominant Hodgkin lymphoma.** Lymph node biopsy showing typical "popcorn cells" (arrows) with a background of small lymphocytes and histiocytes.



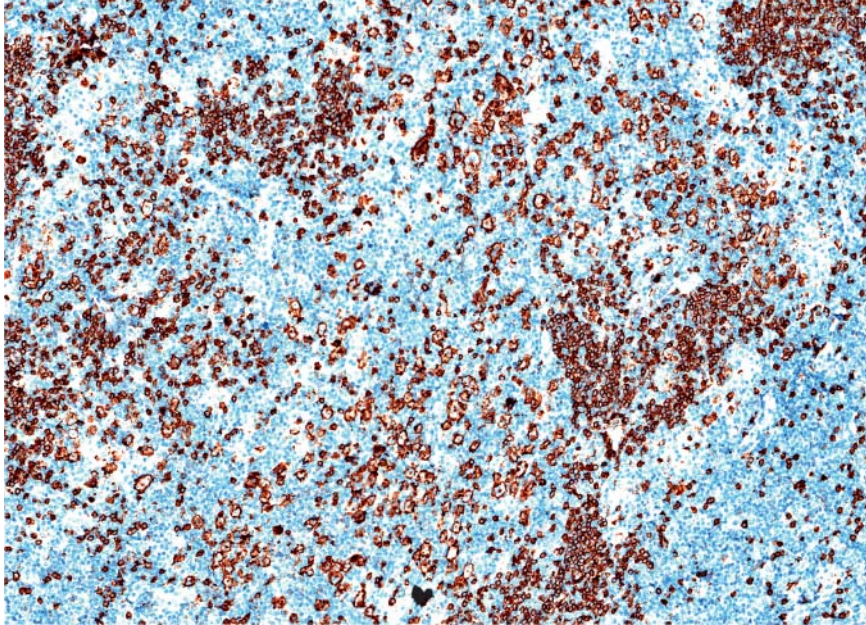


**Fig. 11-1C: Nodular lymphocyte predominant Hodgkin lymphoma.** Lymph node biopsy showing sheets of large B-cell, consistent with nodular lymphocyte predominant Hodgkin lymphoma transformed to diffuse large B-cell lymphoma.

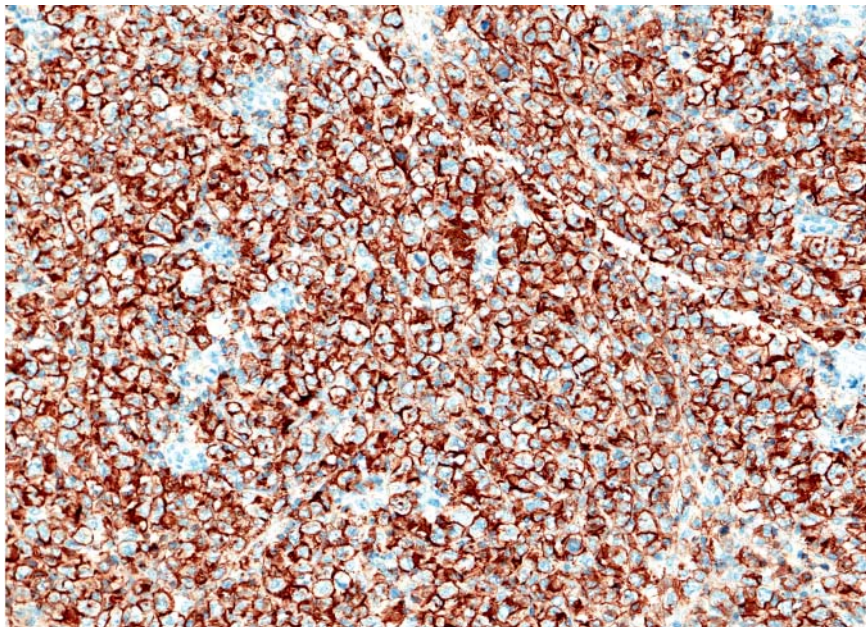


**Fig. 11-1D: Nodular lymphocyte predominant Hodgkin lymphoma.** Immunohistochemical stain for CD20 shows the diffusely stained large B-cell lymphoma area (D) and nodular lymphocyte predominant Hodgkin lymphoma area (N) in the same lymph node (Lymph node biopsy).



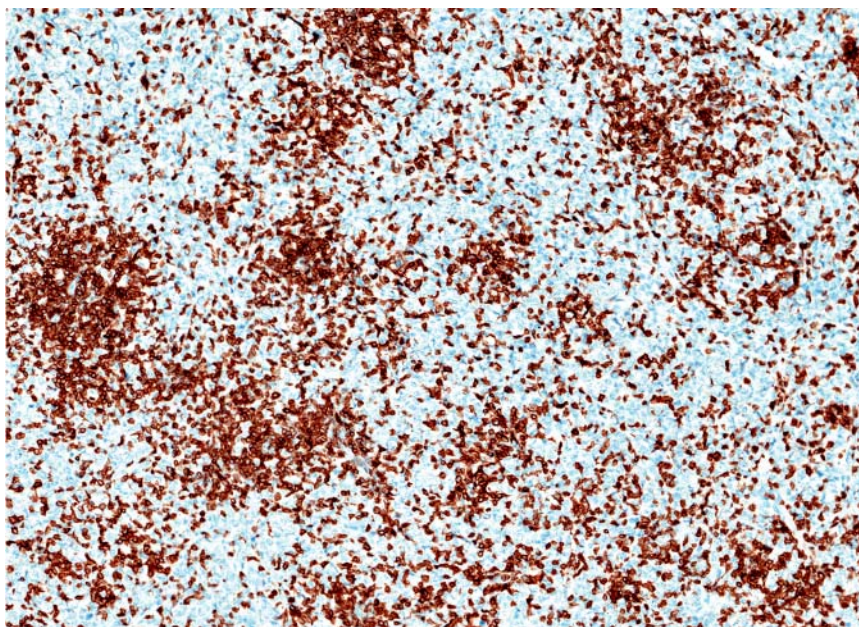


**Fig. 11-1E: Nodular lymphocyte predominant Hodgkin lymphoma.** Immunohistochemical stain for CD20 (high magnification) in the nodular area shows scattered clusters of large CD20-positive "popcorn cells" in a nodular pattern (Lymph node biopsy).



**Fig. 11-1F: Nodular lymphocyte predominant Hodgkin lymphoma.** Immunohistochemical stain for CD20 (high magnification) in the transformed area shows sheets of large CD20-positive cells (Lymph node biopsy).





**Fig. 11-1G: Nodular lymphocyte predominant Hodgkin lymphoma.** Immunohistochemical stain for CD3 shows scattered T-cells. These T-cells can surround the "popcorn cells" and form a rosette-like pattern (Lymph node biopsy).

**TABLE  
11-2**

**Immunophenotype comparison of LP cell and Reed-Sternberg (R-S) cells**

	LP cell	R-S cell
CD20	+	-/+ (variable 20%)
PAX-5	+	+ (weak)
CD45	+	-
CD30	-	+
CD15	-	+/-
BCL2	-	+
BCL6	+	-/+
Fascin	-	+
EMA	+	-
Oct2	+	-/+
BOB.1	+	-

male predominance in all classical Hodgkin lymphoma subtypes except nodular sclerosis, which has an equal frequency in both sexes.

Reed-Sternberg cells are germinal center derived B-lymphocytes. The immunophenotype comparison is listed in Table 11-2.

Adverse biological prognostic factors are CD15-, CD20+, CD68+, BCL2+, and EBV+ in patients >45 years old.

***Nodular Sclerosis Classical Hodgkin Lymphoma***

Nodular sclerosis classical Hodgkin lymphoma (NSCHL) is the most common subtype of classical Hodgkin lymphoma. Bands of collagen divides the lymph node into nodules. Classical Reed-Sternberg cells are rare. A Reed-Sternberg variant, **lacunar cells (due to an artifact of formalin fixed tissue)** are more commonly observed in this subtype. The “**Syncytial variant**” has aggregates of lacunar cells associated with necrosis and histiocytic infiltration (*see* Figs 10-2A to G).

***Mixed Cellularity Classical Hodgkin Lymphoma***

Mixed cellularity classical Hodgkin lymphoma (MCCHL) comprises 15-30% of all classical Hodgkin lymphoma. This subtype is not associated with a bimodal age distribution and is more frequently seen in patients with HIV infection.

Mixed cellularity classical Hodgkin lymphoma has features similar to nodular sclerosis classical Hodgkin lymphoma but without evidence of thick capsule, or thick band-like sclerosis. Classical Reed-Sternberg cells are common and associated with a mixed cellular infiltrate. More than 75% of the cases are EBV+.

Differential diagnosis includes infectious mononucleosis, T cell /histiocyte rich large B-cell lymphoma and peripheral T-cell lymphoma. Cases that do not fit into other subtypes are put into this category (*see* Figs 10-3A and B).

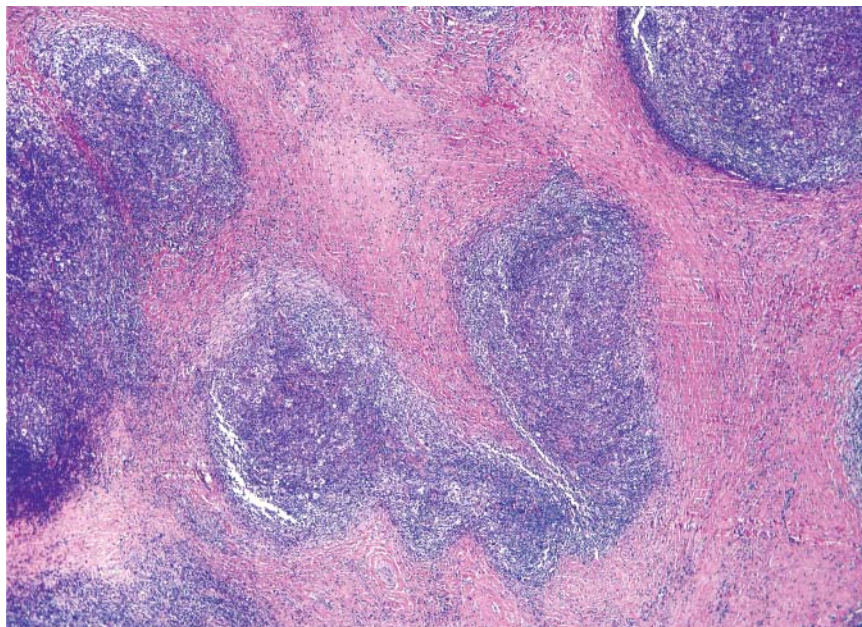
***Lymphocyte Rich Classical Hodgkin Lymphoma***

Lymphocyte rich classical Hodgkin lymphoma (LRCHL) comprises 6% cases of all classical Hodgkin lymphoma. Morphology shows classical or mononuclear variants of Reed-Sternberg cells with a background of small lymphocytes. **Eosinophils and neutrophils are usually absent.** There are two growth patterns: nodular (common) and diffuse (rare). Immunohistochemical stains will distinguish lymphocyte rich classical Hodgkin lymphoma with a nodular growth pattern from nodular lymphocyte predominant Hodgkin lymphoma. The small lymphocytes in the nodules have an IgM+/IgD+ mantle cell phenotype (Table 11-3).

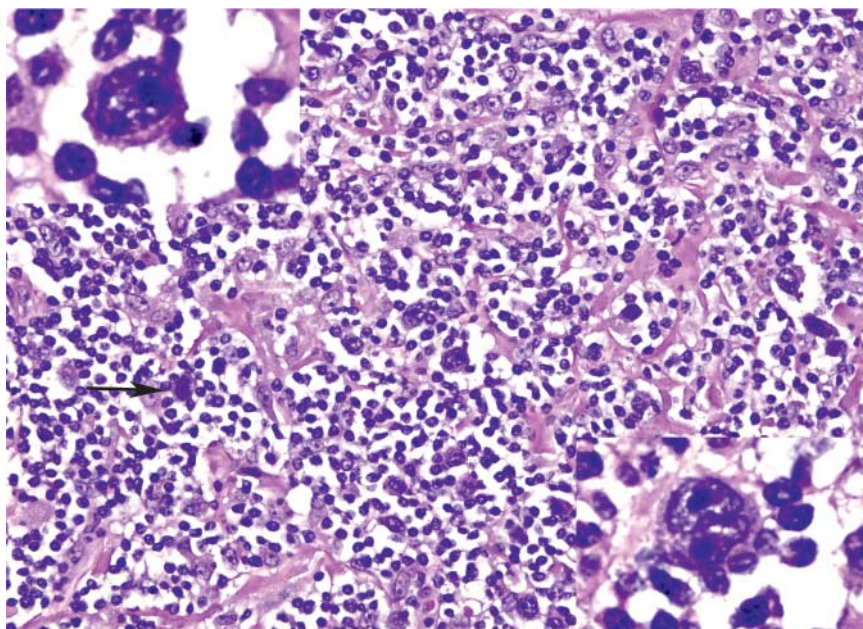
***Lymphocyte-depleted Classical Hodgkin Lymphoma***

Lymphocyte-depleted classical Hodgkin lymphoma (LDCHL) comprises less than 5% cases of all classical Hodgkin lymphoma. Morphology shows a predominance of Reed-Sternberg cells in relation to the background



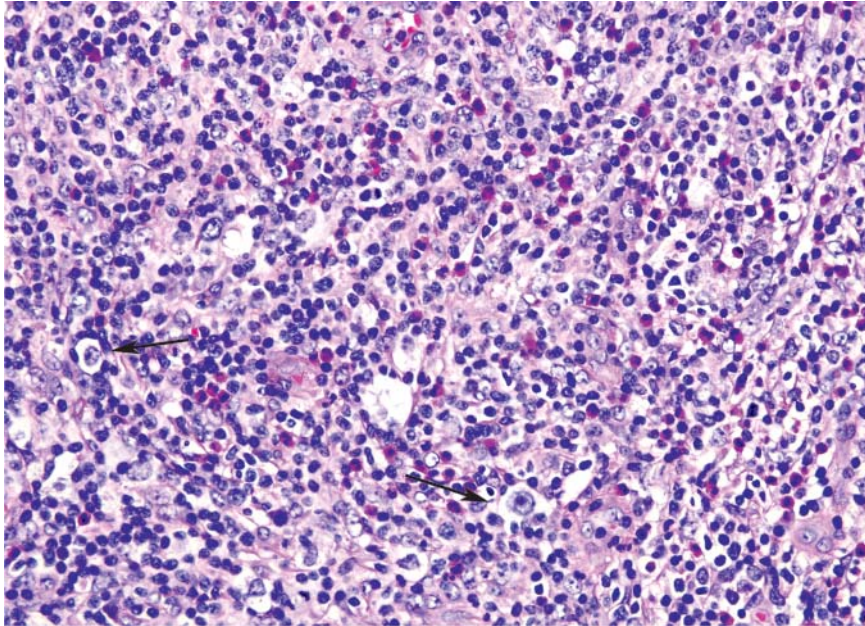


**Fig. 11-2A: Classical Hodgkin lymphoma, nodular sclerosis variant.** Lymph node biopsy showing fibrous bands dividing the lymph node into smaller nodules.

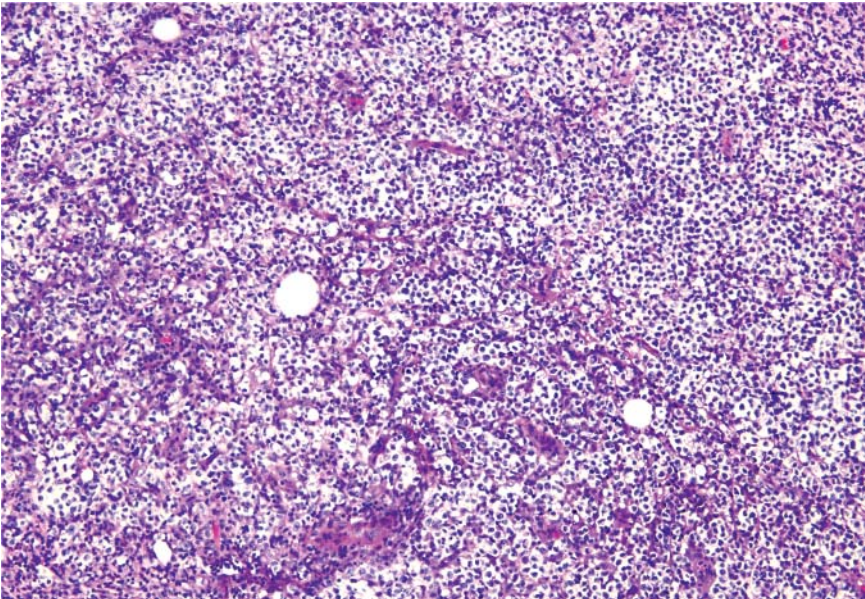


**Fig. 11-2B: Classical Hodgkin lymphoma, nodular sclerosis variant.** Lymph node biopsy showing the typical Reed-Sternberg cell, binucleated and mononuclear variant Reed-Sternberg cell (two insets), and a mummified Hodgkin cell (arrow) in a background of small lymphocytes, plasma cells and eosinophils.



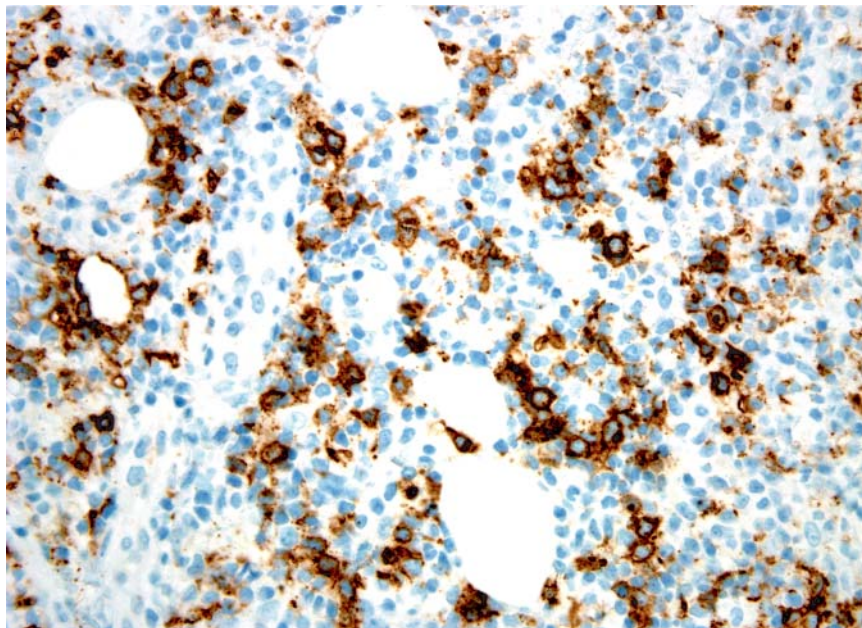


**Fig. 11-2C: Classical Hodgkin lymphoma, nodular sclerosis variant.** Lymph node biopsy showing the typical Reed-Sternberg cell, lacunar variant. Reed-Sternberg cell (arrow) in a background of small lymphocytes, plasma cells and eosinophils. The lacunar cells, an artifact of formalin fixation, are commonly seen in the nodular sclerosis subtype of classical Hodgkin lymphoma.

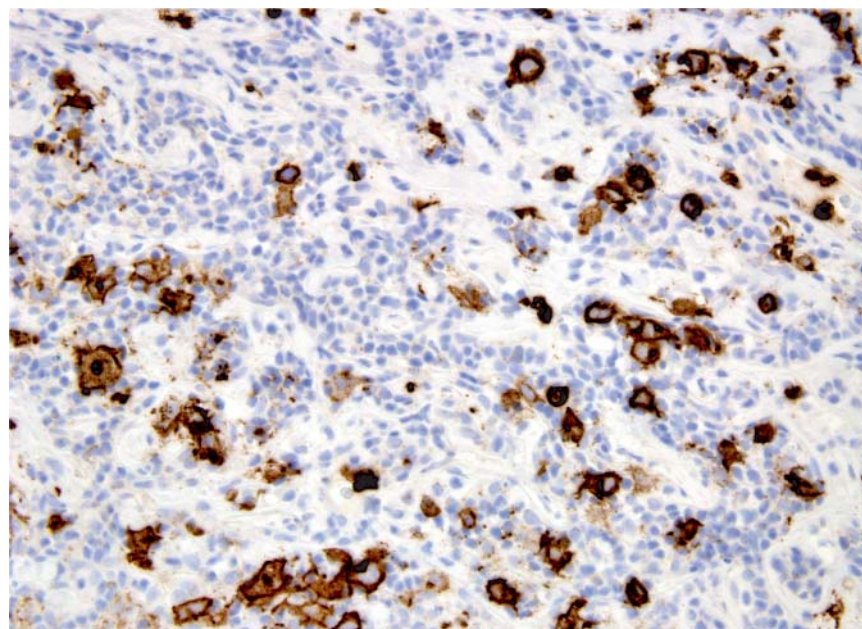


**Fig. 11-2D: Classical Hodgkin lymphoma, nodular sclerosis variant.** The syncytial variant has sheets or aggregates of lacunar variant Reed-Sternberg cells (Lymph node biopsy).

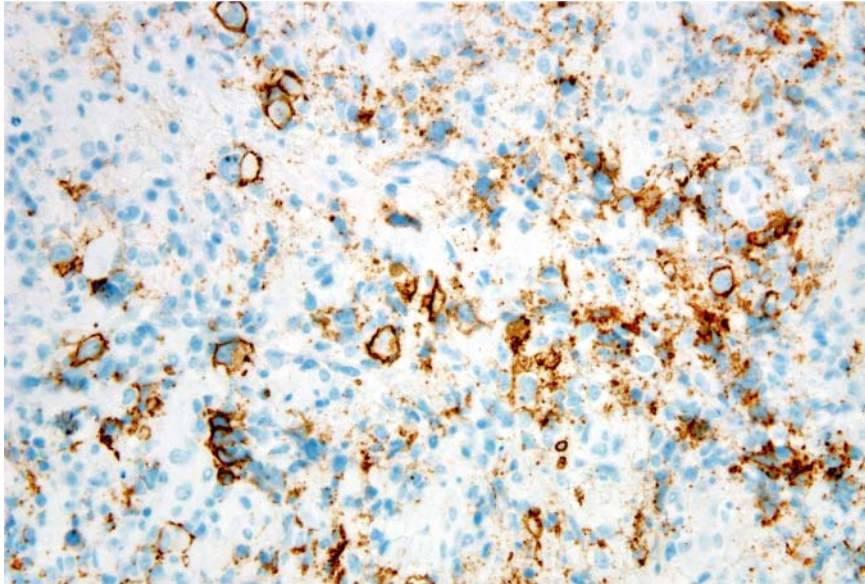




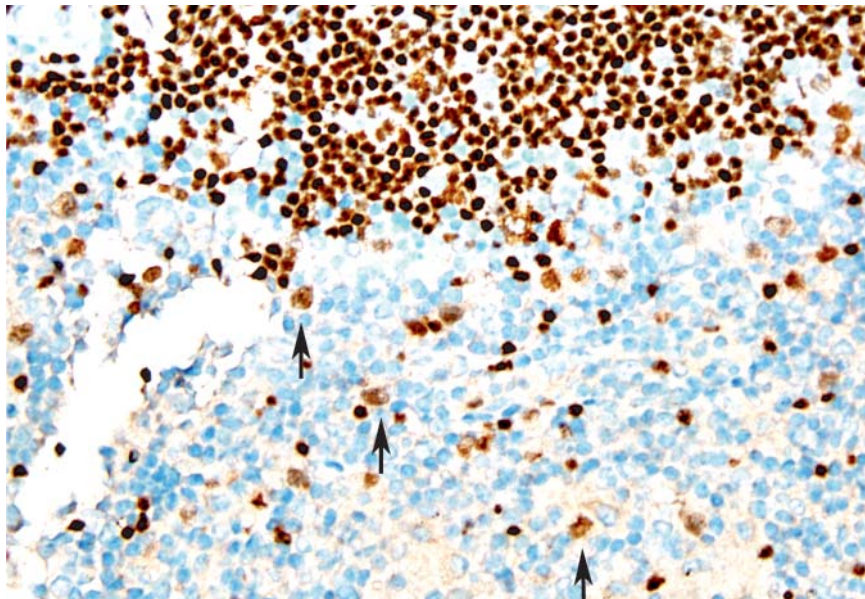
**Fig. 11-2E: Classical Hodgkin lymphoma, nodular sclerosis variant.** Immunohistochemical stain for CD30 shows that the Reed-Sternberg cell has a membrane and paranuclear dot-like (Golgi) stain pattern (Lymph node biopsy).



**Fig. 11-2F: Classical Hodgkin lymphoma, nodular sclerosis variant.** Immunohistochemical stain for CD15 shows the Reed-Sternberg cell has a membrane and paranuclear dot-like stain pattern with a scattered background of small granulocytes (eosinophils and neutrophils) (Lymph node biopsy).

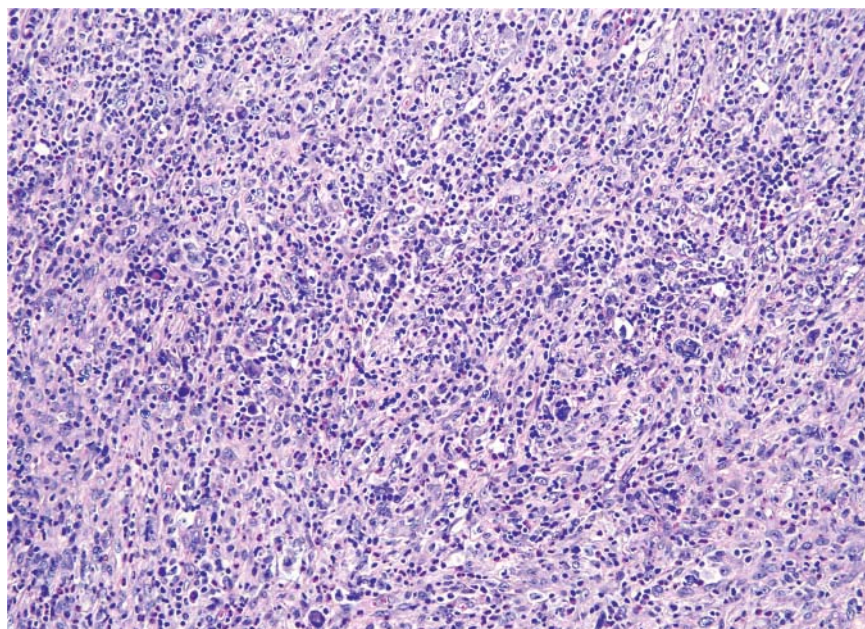


**Fig. 11-2G: Classical Hodgkin lymphoma, nodular sclerosis variant.** Immunohistochemical stain for CD20 shows the Reed-Sternberg cell has a membranous staining pattern. CD20 positive Reed-Sternberg cells are only present in a small number of cases (Lymph node biopsy).

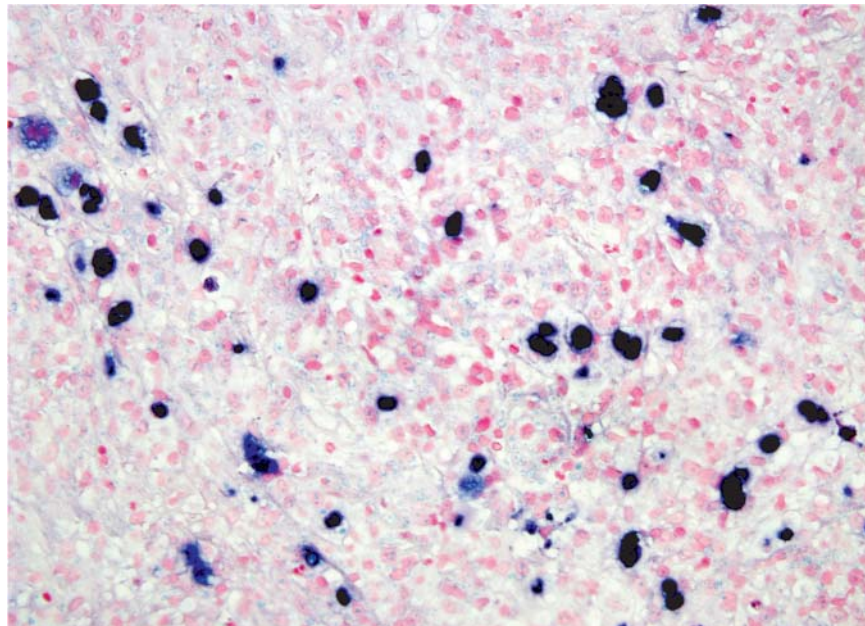


**Fig. 11-2H: Classical Hodgkin lymphoma, nodular sclerosis variant.** Immunohistochemical stain for PAX-5 shows the Reed-Sternberg cell has a weaker nuclear staining pattern than the surrounding intensely stained normal B lymphocytes. PAX-5 is very helpful in differentiating classical Hodgkin lymphoma from ALK-1 negative ALCL (Lymph node biopsy).





**Fig. 11-3A: Classical Hodgkin lymphoma, mixed cellularity variant.** Lymph node biopsy showing effacement of normal architecture with scattered Reed-Sternberg cells in a background of small lymphocytes, plasma cells and eosinophils (Lymph node biopsy).



**Fig. 11-3B: Classical Hodgkin lymphoma, mixed cellularity variant.** In situ hybridization for EBV shows strong expression of EBV positive Reed-Sternberg cells. This variant has a higher EBV positive frequency than NSCHL and LRCHL (approximately 75% positive) (Lymph node biopsy).

lymphocytes. Co-expression of CD30 and PAX-5 (weakly positive) is very helpful in differentiating classical Hodgkin lymphoma from ALK negative anaplastic large cell lymphoma. Lymphocyte-depleted subtype is often associated with HIV infection.

### Composite Hodgkin Lymphoma

Hodgkin lymphomas may co-exist with other non-Hodgkin lymphomas (composite lymphoma).

Classical Hodgkin lymphoma may coexist with follicular lymphoma, SLL/CLL, mantle cell lymphoma, marginal zone lymphoma, and diffuse large B-cell lymphoma.

Nodular lymphocyte predominant Hodgkin lymphoma may coexist with diffuse large B-cell lymphoma.

**TABLE  
11-3**

**Immunophenotype comparison of classical Hodgkin lymphoma (CHL), nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and anaplastic large cell lymphoma (ALCL)**

	CHL	NLPHD	ALCL, ALK+	ALCL, ALK-
CD45	–	+	+/-	+/-
CD20/CD79a	-/+	+	–	–
CD30	+	–	–	–
CD15	+	–	–	–
PAX-5	+	+	–	–
CD2	–	–	-/+	+/-
CD3	–	–	-/+	-/+
Perforin/Granzyme B	–	–	+	+
ALK	–	–	+	–
Oct2	-/+	+	not apply	not apply
BOB.1	–	+	not apply	not apply
EBV	+/-	–	–	–

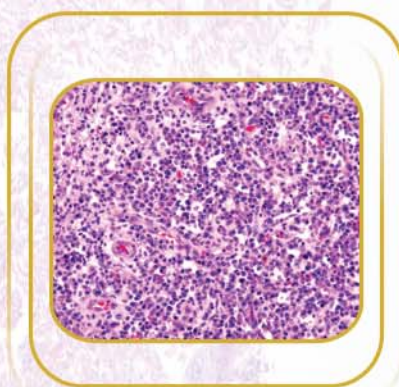




CHAPTER

12

# **Lymphoproliferative Disorders Associated with Immunodeficiency and Post-transplantations**



## *Primary Immune Disorders Associated with Lymphoproliferative Disorders*

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### **Wiskott-Aldrich Syndrome**

Wiskott-Aldrich syndrome is a rare X-linked disorder characterized by thrombocytopenia, small platelets, eczema, recurrent infections, immunodeficiency, and a high incidence of autoimmune diseases and malignancies.

The WAS gene (**Xp11.22**), which encodes the Wiskott-Aldrich syndrome protein (WASP) is mutated in Wiskott-Aldrich syndrome patients. WASP is the key regulator of hematopoietic cells and is involved in cell signaling, locomotion, and immunologic synapse formation. Mutation of this gene results in reduced expression of CD43 on the lymphocytes. Flow cytometry analysis of peripheral blood mononuclear cell surface CD43 expression is a helpful tool to diagnose Wiskott-Aldrich syndrome. Wiskott-Aldrich syndrome patients show a reduced level of serum IgM, elevated level of serum IgA and IgE, and a normal level of IgG.

Approximately 10-20% of Wiskott-Aldrich syndrome patients develop lymphoma, most commonly diffuse large B-cell lymphoma (immunoblastic variant).

### **Common Variable Immunodeficiency Disorder**

Common variable immunodeficiency disorder (CVID) is a heterogeneous immunodeficiency disorder clinically characterized by an increased incidence of recurrent infections, autoimmune phenomena and neoplasms. The onset is usually during adolescence or early adulthood but can occur at any age. It is the most common cause of pan-hypogammaglobulinemia in adults. Sporadic cases are most common; however, there are several models of inheritance, including autosomal recessive, autosomal dominant and X-linked. Most patients are of European descent. Males and females are equally affected.

Extranodal reactive **lymphoid hyperplasia** is common, followed by **atypical lymphoid hyperplasia**, **chronic granulomatous inflammation**, and **malignant lymphoma** (1-7% of cases progress to malignant lymphoma).

### **Ataxia Telangiectasia**

Ataxia telangiectasia is an autosomal recessive multisystem disorder due to mutations in the AT gene that involves DNA repair. Ataxia telangiectasia is characterized by immunodeficiency, progressive neurologic impairment and oculocutaneous telangiectasia.

The immune deficiency is variable and involves both cellular and humoral immunity. The majority of ataxia telangiectasia patients have low or absent IgA and IgE. Infection is the most common causes of death followed by malignancies. The majority of malignancies are lymphomas, leukemias and solid tumors (adenocarcinoma, dysgerminoma, gonadoblastoma, and medulloblastoma). Unlike other immunodeficiency disorders, the lymphomas and leukemias in ataxia telangiectasia patients are predominantly of T-cell origin.

### **X-linked Lymphoproliferative Syndrome**

Patients are extremely susceptible to EBV infections leading to uncontrolled expansion of EBV-infected B-cells and CD8+ cytotoxic T-cells. As would be expected, nearly 100% of the associated lymphoproliferative disorders are EBV-associated lesions.

### ***Disorders Associated with Acquired Immunodeficiency***

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#### **Lymphomas that Associated with HIV Infection**

1. Primary effusion lymphoma: Usually only express CD45. Some cases also express plasma cell-related antigens (CD30, CD38 and CD138). The malignant cells are KSHV/HHV8+ and EBV+.
2. Plasmablastic lymphoma of the oral cavity: CD45+ and CD138+, half of the cases are EBV+. KSHV/HHV8 is usually negative.
3. Burkitt lymphoma.
4. Diffuse large B-cell lymphoma.
5. Extranodal marginal zone lymphoma.
6. Peripheral T-cell lymphoma.
7. Classical Hodgkin lymphoma.
8. Polymorphic B-cell PTLTD.

#### **Multicentric Castleman's Disease with HIV Infection**

Poor prognosis, frequently involves lymph nodes and spleen.

### ***Post-transplant Lymphoproliferative Disorder***

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Post-transplant lymphoproliferative disorder (PTLD) results from lymphoid or plasmacytic proliferations that develop in the setting of solid organ or

bone marrow transplantation. PTLD represents an uncommon complication in transplant patients. Major risk factors identified with the development of PTLD include pretransplant EBV-positive serology, the type of organ transplanted, and the intensity of immunosuppressive regimen used. The incidence of PTLD is approximately 1 to 2% of solid-organ transplant recipients. There is a clear association between PTLD and the type of organ transplanted. Among solid organ recipients, those with **cardiac-lung** or **intestinal** transplant has the **highest** incidence of PTLD. **Kidney** or **bone marrow** transplant has the **lowest** incidence of PTLD. The onset of posttransplant lymphoma in most patients is related to B-cell proliferation induced by an EBV infection in the setting of chronic immunosuppression. The majority (>90%) of cases of PTLD in solid organ transplant are of host origin with only a minority being of donor origin. However, in bone marrow transplant recipients, the majority of PTLD are of donor origin. PTLD commonly involves lymph nodes, GI tract, lung and liver. Involvement of the CNS is rare.

Management of PTLD includes decreasing the dose of immunosuppressive drugs, antiviral therapy, interferon, intravenous immunoglobulin, adoptive therapies with EBV-specific cytotoxic T-lymphocytes, chemotherapy, radiation, and rituximab therapy.

### Classification of Post-transplant Lymphoproliferative Disorders (WHO 2008)

1. Early lesions
  - a. Plasmacytic hyperplasia
  - b. Infectious mononucleosis-like polyclonal proliferation.
2. PTLD.
3. Polymorphic PTLD.
4. Monomorphic PTLD (B- and T/NK-cell types).
5. Classical Hodgkin lymphoma type PTLD.

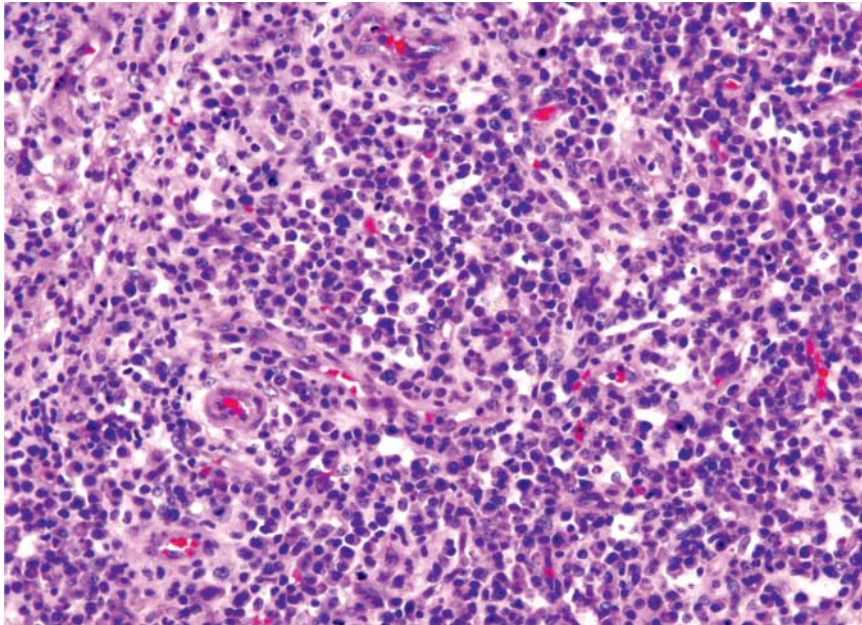
### Diagnostic Approach to PTLD

1. Histologic evaluation of architecture and cytological features.
2. Immunophenotyping by immunohistochemical and flow cytometry studies.
3. In situ hybridization for EBV (EBER).
4. Gene rearrangement and cytogenetic studies.

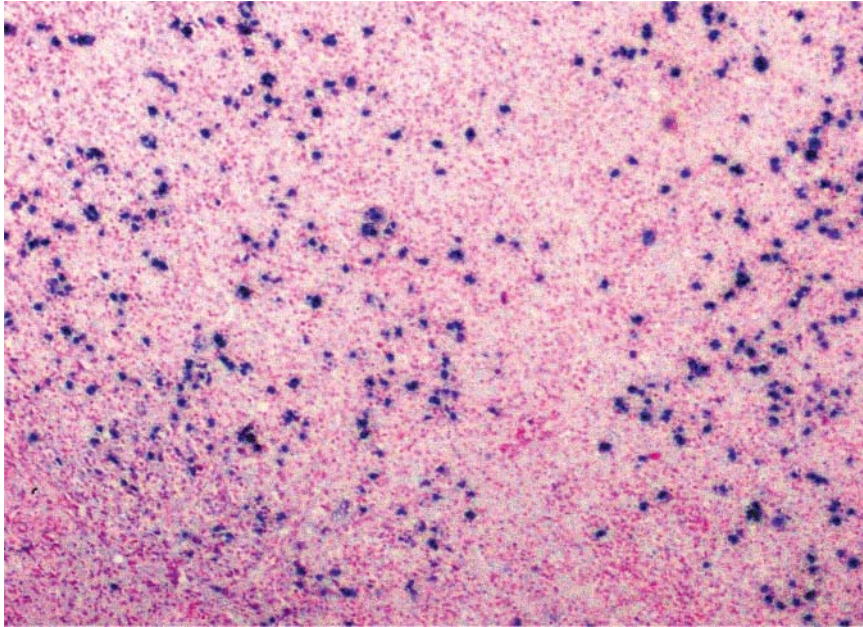


## Prognosis

1. Early lesions tend to regress with reduction of immunosuppression therapy.
2. Polymorphic, and less commonly monomorphic proliferations may regress with the reduction of immunosuppression but frequently need additional anti-B-cell or chemotherapy.
3. Myelomatous lesions usually do not regress with reduction of immune suppression therapy.
4. T/NK-cell types PTLD are rare and usually aggressive (approximately 100 confirmed cases).
5. BCL-6 gene mutation (3q27) in PTLD is associated with a poor prognosis (Figs 12-1A and B).



**Fig. 12-1A: Post-transplant lymphoproliferative disorder (PTLD), polymorphous type.** Lymph node biopsy showing a replacement of the normal architecture by a mixture of small lymphocytes, larger activated lymphocytes, and plasma cells. Flow cytometry was negative for a monoclonal B-cell population. In situ hybridization showed a polyclonal plasma cells population.



**Fig. 12-1B: Post-transplant lymphoproliferative disorder (PTLD), monomorphous type.** In situ hybridization for EBER shows the cells to be EBV positive. Flow cytometry was positive for a monoclonal B-cell population.

A large, light purple histological section of tissue, likely a skin biopsy, serves as the background for the entire page. It shows various cellular structures and layers. A red horizontal bar is positioned across the middle of the page, partially obscuring the tissue image. On the left side of this bar, there are three small, overlapping squares in green, blue, and yellow, each with a thin black outline.

CHAPTER

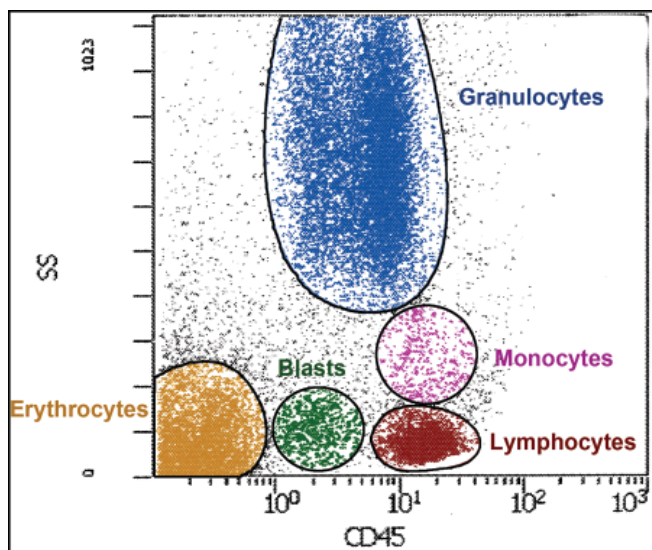
13

# Flow Cytometry

### *Principles of Flow Cytometry and its Applications*

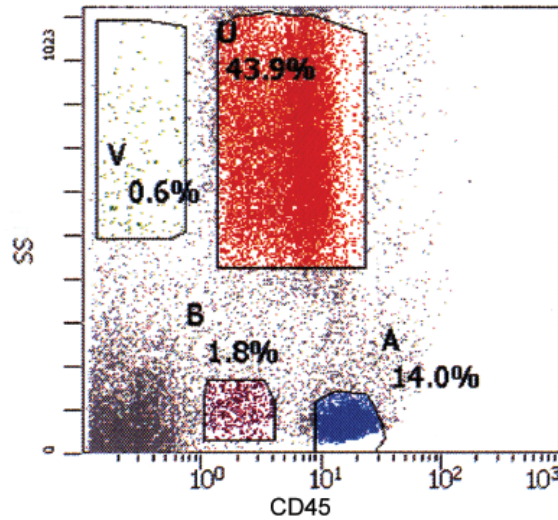
The basic principle of flow cytometry (FCM) is based on creating a fluid suspension of cells and then passing through a small capillary tube. A light beam (usually laser) is then directed on the tube. Cells passing through the tube will scatter the light, which is collected by detectors positioned at different angles around the capillary tube. As a cell passes through the tube (and laser beam) multiple parameters can be measured regarding the **cell's size (Forward Scatter, FSC)**, **nuclear complexity/cytoplasmic granularity (Side Scatter, SSC)**, and surface markers.

Gating, in which specific population of cells can be identified, is the first step in the interpretation of flow cytometry. The standard scatter plot used for initial assessment of hematopoietic cells compares side scatter and expression of CD45. This is primarily used to identify a population of blasts (dim expression of CD45, and low side scatter) present within a heterogeneous cell population. The percentage of the subpopulation of cells accounting for the entire cell population is determined by gating (Figs 13-1A and B).



**Fig. 13-1A: Flow cytometry.** Side scatter versus CD45 dot plot showing the relative locations of lymphocytes, blasts, monocytes, granulocytes and erythrocytes populations (Normal bone marrow aspirate).





**Fig. 13-1B: Flow cytometry.** Side scatter versus CD45 dot plot showing gating information of the percentage of (A) lymphocytes, (B) blasts, and (U) granulocytes (Bone marrow aspirate).

## Applications of Flow Cytometry

1. Diagnosis of hematopoietic neoplasms, immunodeficiency disorders, paroxysmal nocturnal hemoglobinuria, and lineage phenotyping
2. Analysis of DNA ploidy/cell cycle
3. CD4 T-cell count
4. CD34 stem cell count
5. Reticulocyte count
6. Fetal red blood cell count
7. Anti-platelet antibodies (screening test).

## Lineage Associated Markers of Flow Cytometry Phenotyping

1. The lineage marker **B-cell** is **cCD22**. CD19 and CD10 may also be expressed in the early B cells. cCD22+, CD19+, and CD79a+ support a B-cell lineage.
2. The lineage marker of **T-cell** is **cCD3** (cytoplasmic CD3). cCD3 appears in the earliest T-cell precursors. CD2 and CD7 may present on early T-cell precursors.

3. The lineage markers of granulocytes (myeloid) are **CD13**, **CD33** and **CD117**. Promyelocytes do not express CD34 and HLA-DR. Myelocytes and metamyelocytes may express CD15, CD11b and CD16. Intense expression of CD14 is a feature of monocytes.
4. The lineage marker of **erythroid precursors** is down regulated CD45 expression and bright expression of CD71 and CD235a.
5. The marker of **immature monocyte** is CD14 (My4) and the markers of **mature monocyte** are CD11b, CD14 (Mo2), and CD64.
6. The markers of **megakaryocyte** are CD41 and CD61.
7. The markers of **NK cells** are CD16, CD56 and CD57. CD56 is also frequently present in AML, small cell carcinoma and plasma cells. NK cells can be identified based on CD16, CD56 and CD57 expression.
8. Common activation markers are HLA-DR, CD25 and CD30.
9. Aberrant non-lineage expression of markers may also occur, for example aberrant expression of CD7, a T-cell marker, is common in AML.
10. **Mixed lineage leukemia:** Two or more blast populations that express different lineage markers (myeloid blasts and lymphoid blasts).
11. **Mixed phenotype leukemia:** Single blast population expresses different lineage markers.

**TABLE  
13-1****WHO requirement for assigning more than one lineage in a single blast population**

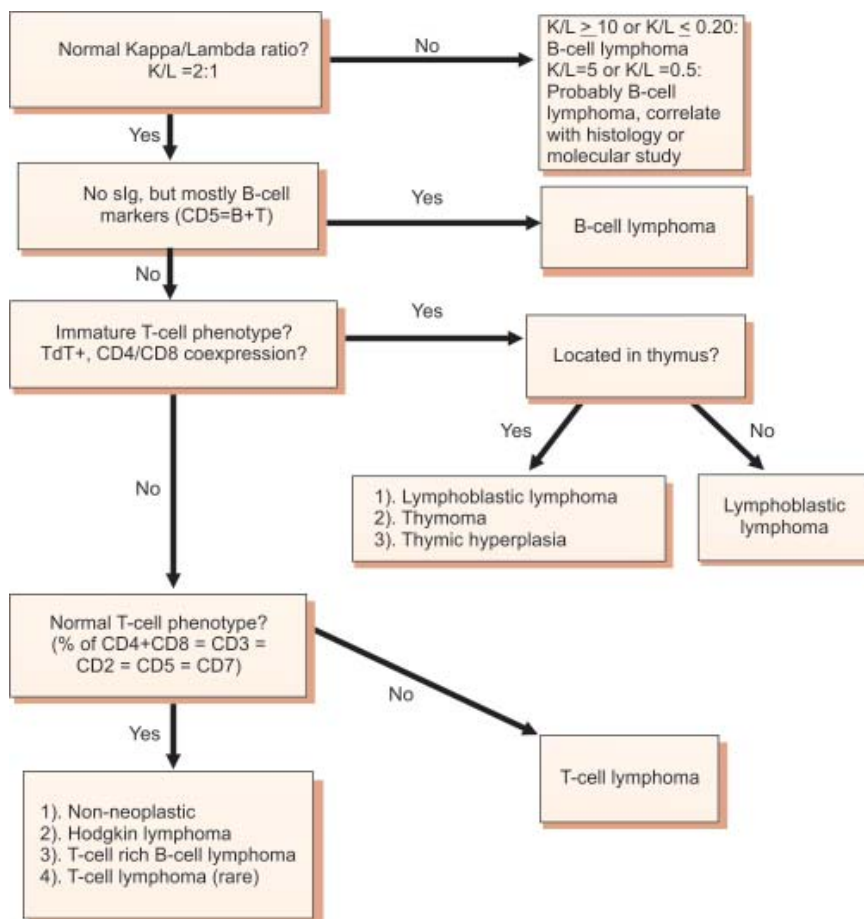
Myeloid	B-cell	T-cell
MPO or Monocytic differentiation (at least two of the following: NSE, CD11c, CD14, CD64, or lysozyme)	Strong CD19 expression with strong expression of at least <b>one</b> of the following: CD79, cCD22, CD10 or Weak CD19 expression with strong expression of at least <b>two</b> of the following: CD79, cCD22, CD10	cCD3 detected by flow cytometry for epsilon chain or surface CD3

## Interpretation Flow Cytometry Results

Morphologic evaluation of the specimen is a critical adjunct to the interpret flow cytometry results.

Assessing fluorescence intensity is an important component in characterizing hematopoietic neoplasms. The expression of CD45 is markedly down regulated

(dim) in some of the acute leukemias such as precursor B acute lymphoblastic leukemia. In side scatter vs. CD45 plot, the blast population is often located in the characteristic blasts region shown in Figures 13-1A and B. In acute myeloid leukemia with monocytic differentiation, the plotted location of blast is dependant on the degree of monocytic differentiation and the relative numbers of the mature monocytes. In cases of acute promyelocytic leukemia, the blast population is usually a single major population with weak to moderate CD45 expression (fluorescence intensity) and a wide range of side-scatter patterns. A flow chart of identifying neoplastic T- and B-cell populations is listed in Figure 13-2.

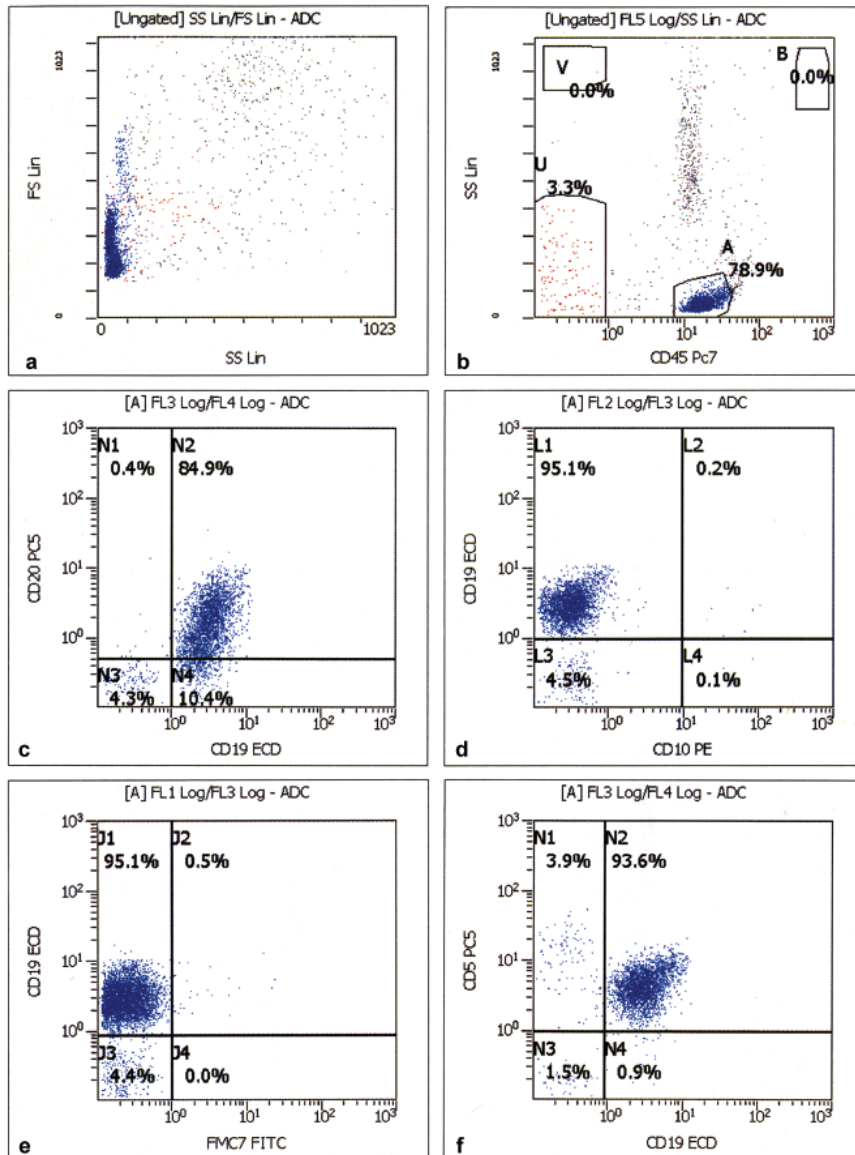


**Fig. 13-2:** Flow chart for identification of neoplastic T- and B-cell populations.

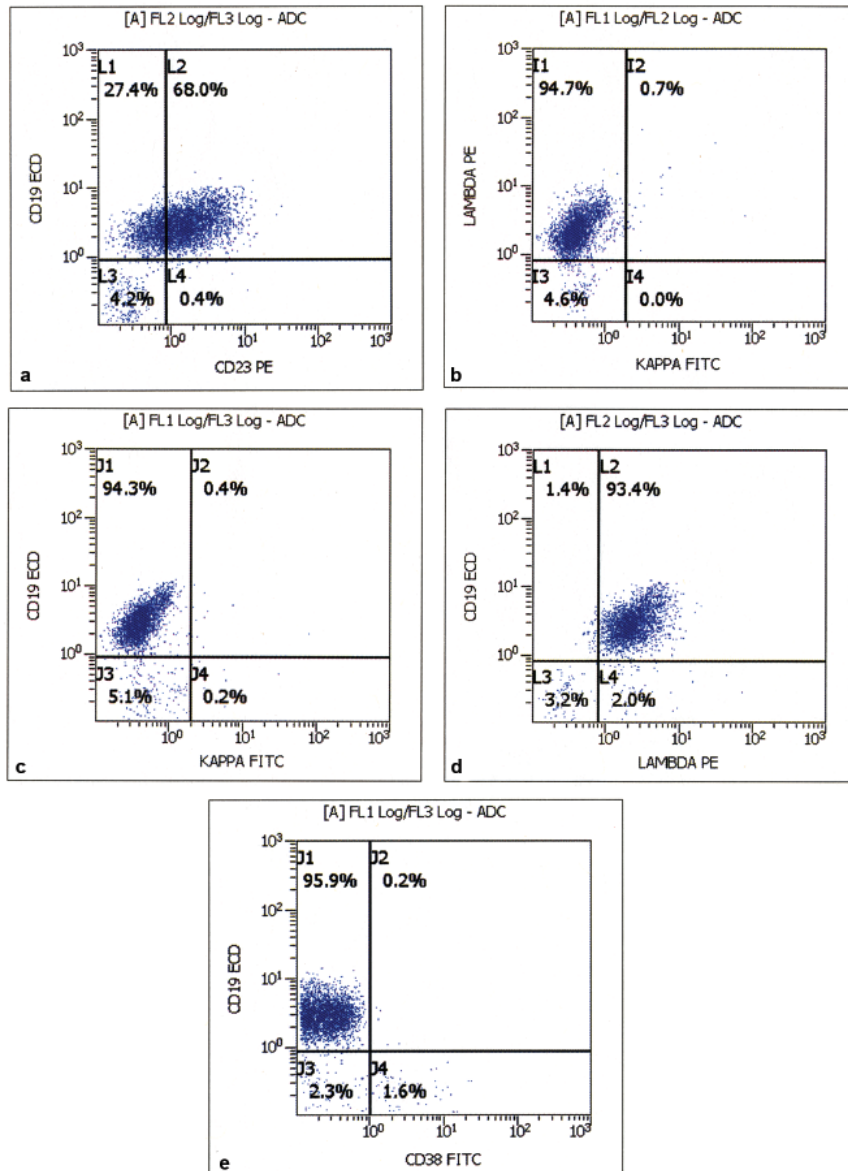
### **Flow Cytometry Study Cases**

- 1. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), peripheral blood (Figs 13-3A to D).**
- 2. B-cell prolymphocytic leukemia (PLL), peripheral blood (Figs 13-4A to C).**
- 3. Mantle cell lymphoma, peripheral blood (Figs 13-5A to C).**
- 4. Mantle cell lymphoma mimics atypical CLL/SLL, lymph node (Figs 13-6A and B).**
- 5. Follicular cell lymphoma, lymph node (Fig. 13-7).**
- 6. Hairy cell leukemia (HCL), peripheral blood (Figs 13-8A and B).**
- 7. Acute myeloid leukemia (AML), bone marrow (Fig. 13-9).**
- 8. Acute myelomonocytic leukemia (AMML), bone marrow (Figs 13-10A and B).**
- 9. Acute promyelocytic leukemia (APL), bone marrow (Fig. 13-11).**
- 10. Acute lymphoblastic lymphoma/leukemia (ALL), mediastinal mass (Figs 13-12A and B).**
- 11. Sézary syndrome, peripheral blood (Figs 13-13A and B).**

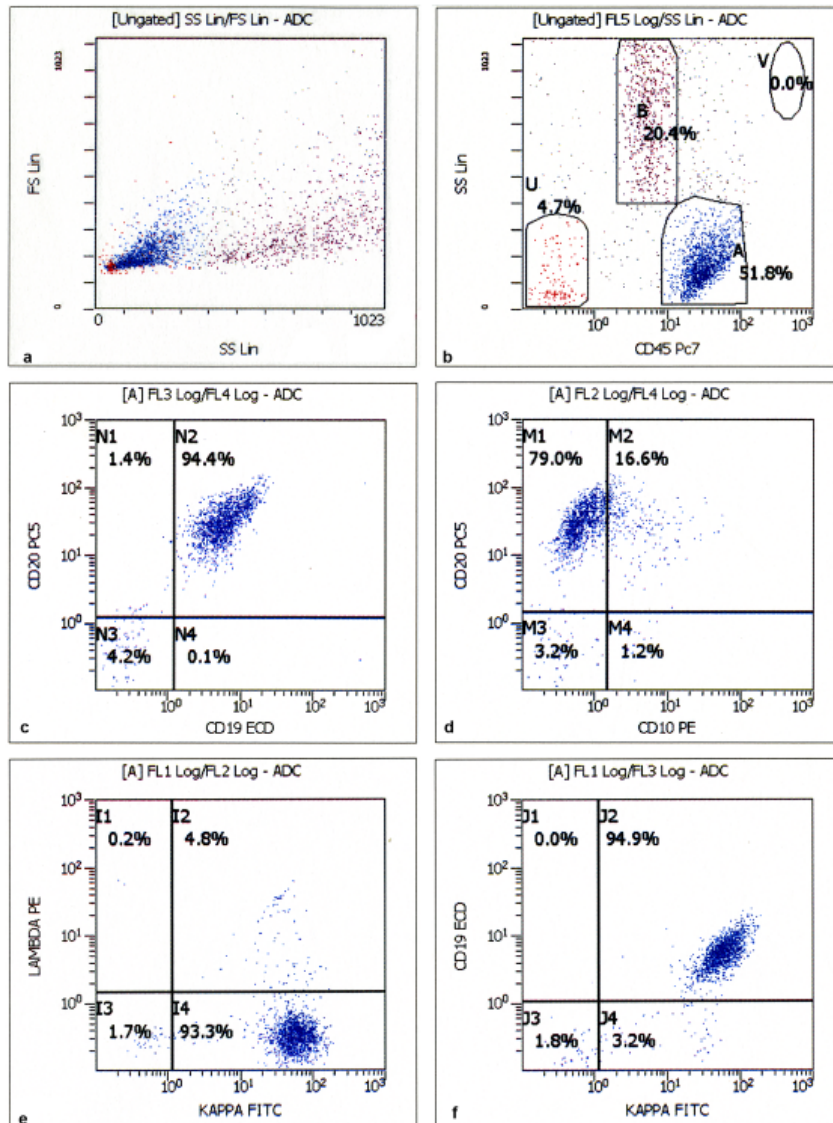




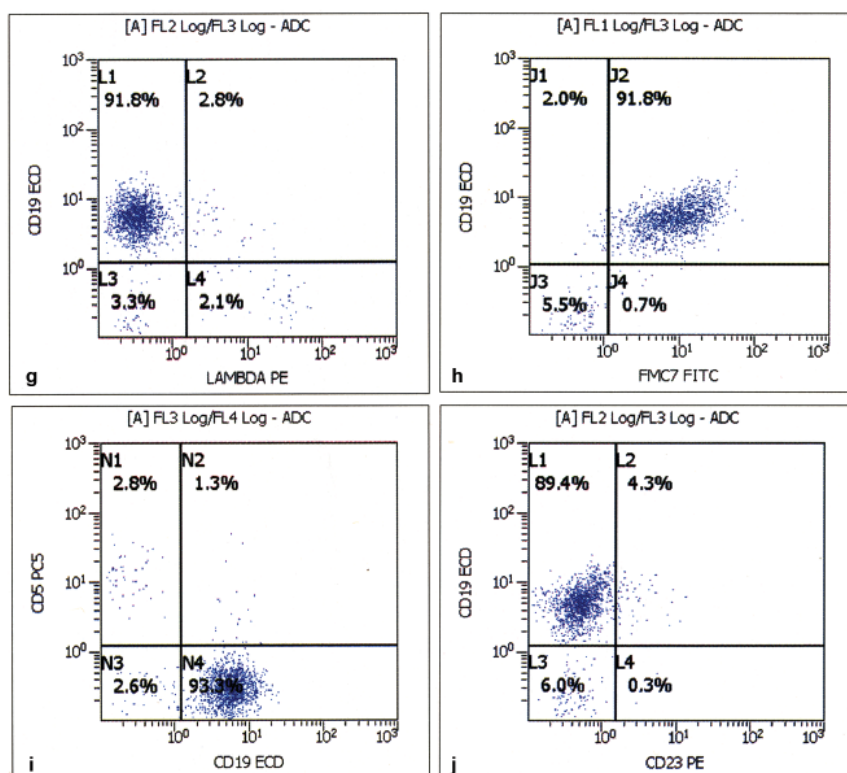
**Fig. 13-3A: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL).** (a) Forward scatter versus side scatter, two clusters of lymphocyte. The predominant cells are small lymphocytes with a minor population of prolymphocytes. (b) CD45 intensity versus side scatter. The intensity of CD45 is usually less than normal lymphocytes. (c) CD20 expression is characteristically weak/dim, and also heterogeneous in comparison to CD19. (d and e) CD10 and FMC7 are negative. (f) Aberrant expression of CD5 and its co-expression with CD19 and CD23 is characteristic (Peripheral blood).



**Fig. 13-3B: Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL).** (a) Co-expression of CD19 and CD23. (b to d) The neoplastic B-cell population is positive for Lambda light chain and negative for Kappa light chain. (e) Expression of CD38 or ZAP-70 is associated with an adverse prognosis; in this case the CD38 is negative (Peripheral blood).

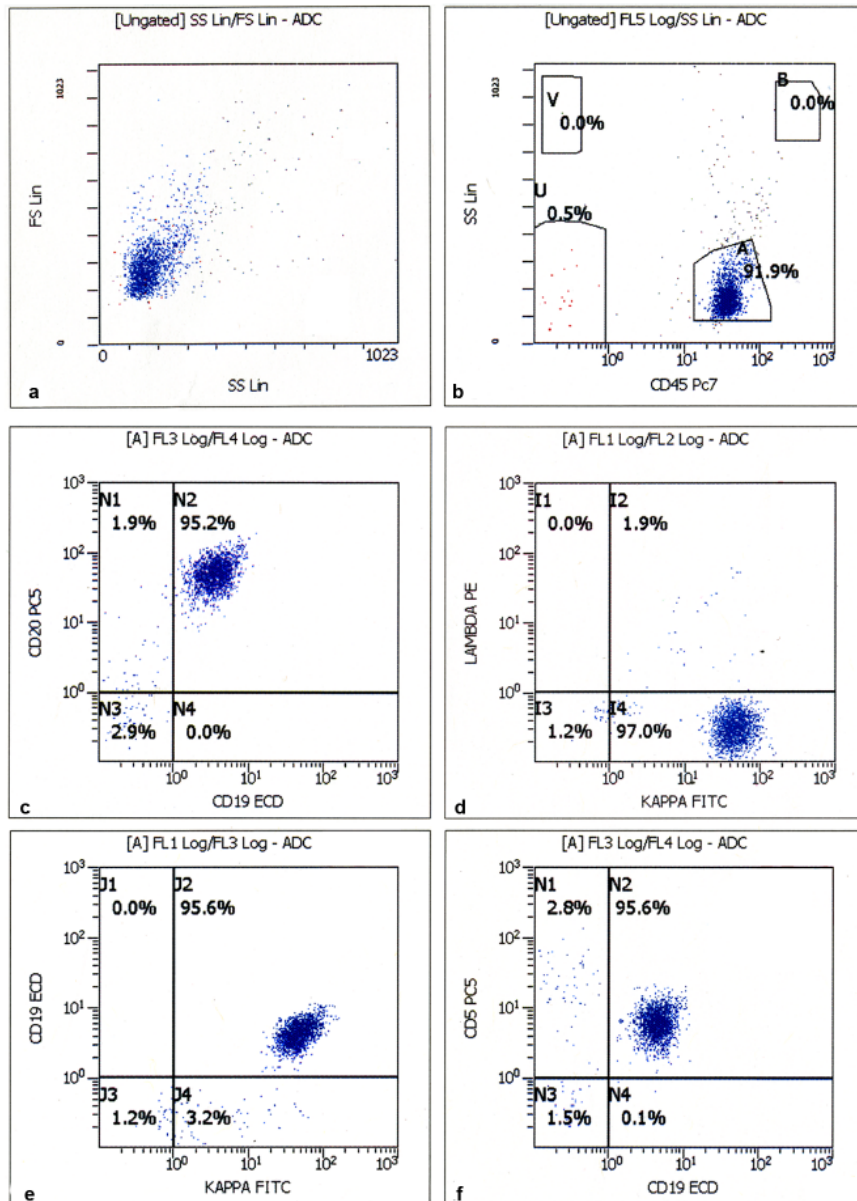


**Fig. 13-4A: B-cell prolymphocytic leukemia (PLL).** (a and b) Lymphocytes comprise 51.8% of the total cell population. (c and d) The expression of CD20 is bright, and CD10 is negative. (e and f) There is clonal expression of Kappa light chain (Peripheral blood).

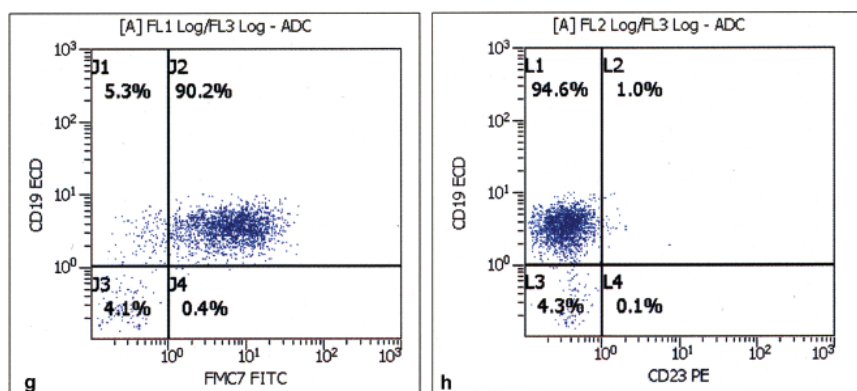


**Fig. 13-4B: B-cell prolymphocytic leukemia (PLL).** (h) The neoplastic B-cells are FMC7 positive. (g, i and j) CD5, CD23, and Lambda light chain are negative (Peripheral blood).

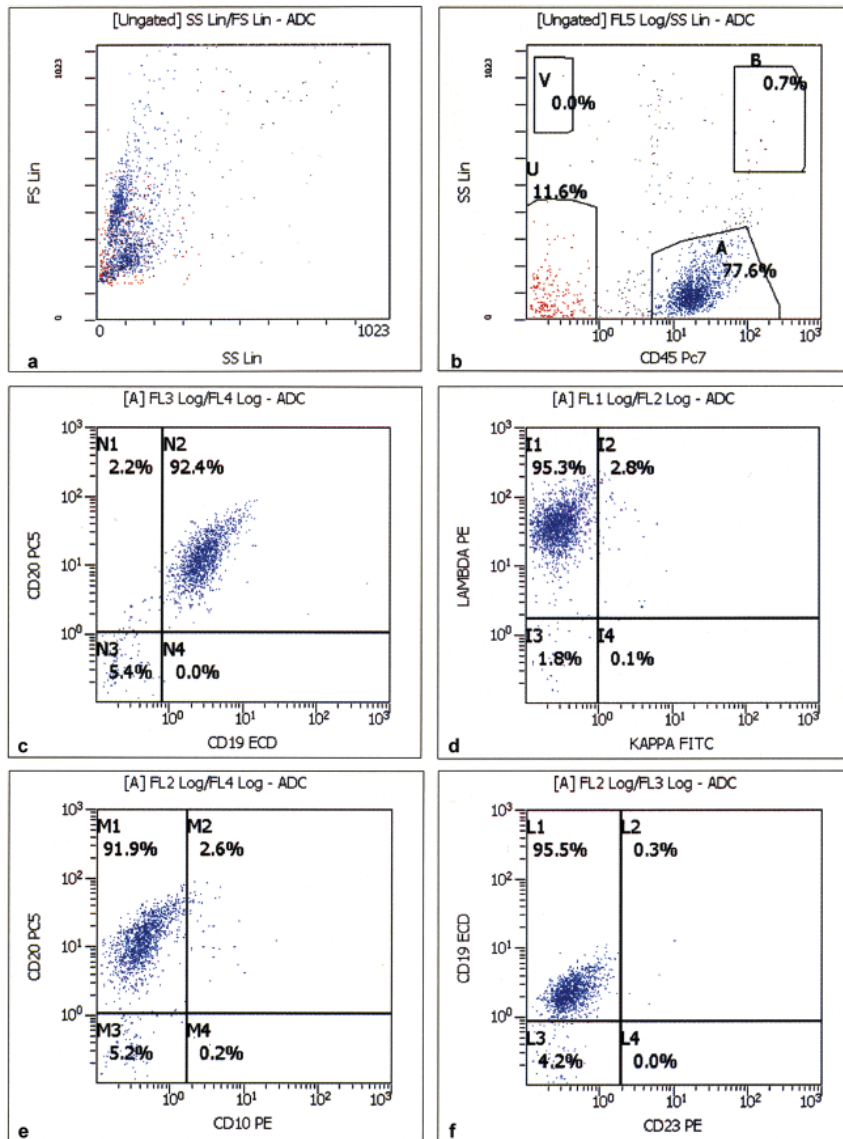




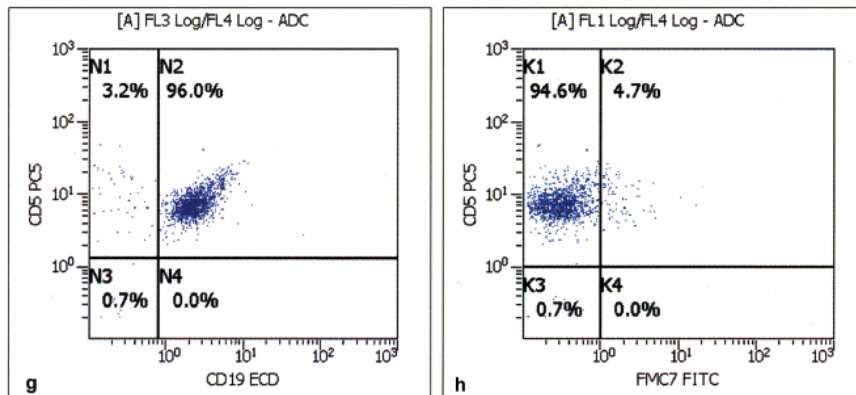
**Fig. 13-5A: Mantle cell lymphoma (MCL).** (a and b) Lymphocytes comprise 91.9% of the total cell population. (c) Bright CD20 expression. (d and e) Clonal Kappa light chain restriction. (f) Aberrant expression of CD5 (Lymph node).



**Fig. 13-5B: Mantle cell lymphoma (MCL).** (g) FMC7 is positive. (h) CD23 is negative (Lymph node).

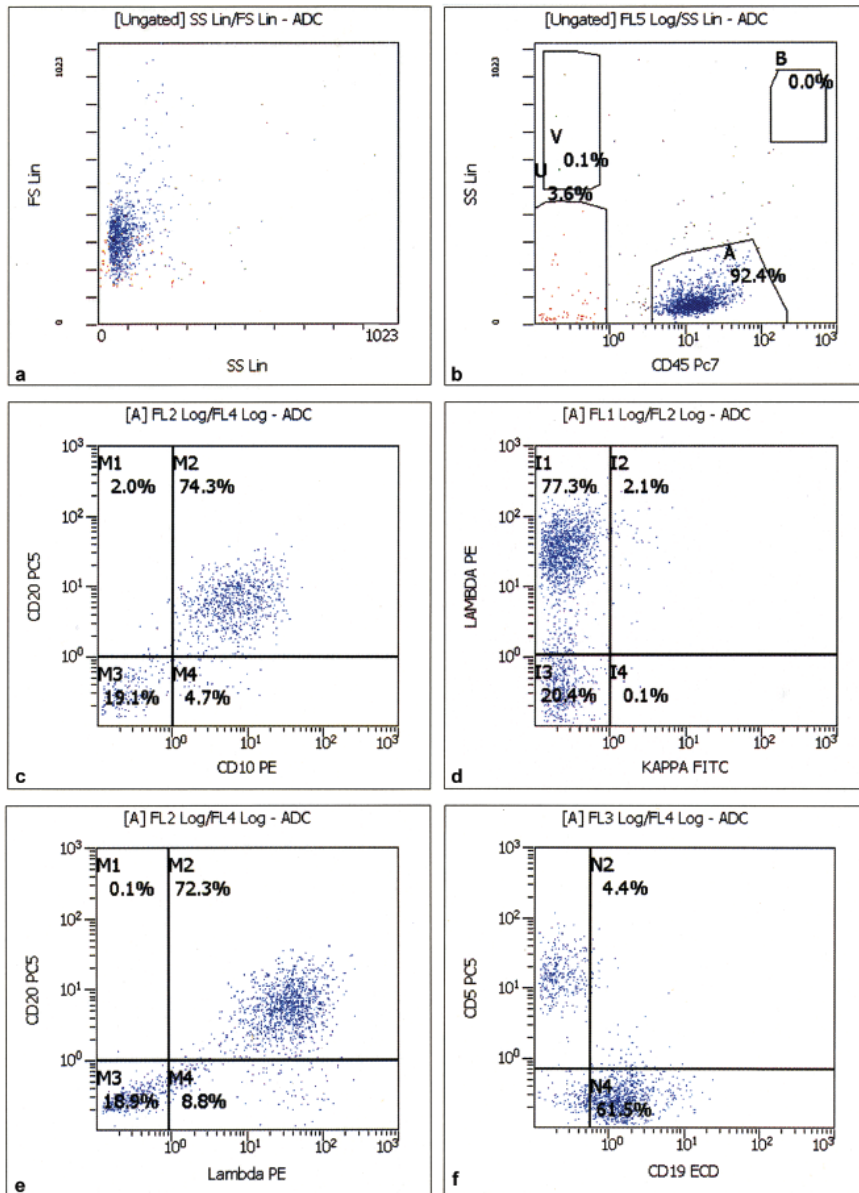


**Fig. 13-6A: Mantle cell lymphoma mimicking atypical CLL/SLL.** (a) Two clusters of small and medium sized lymphocytes. (b) Lymphocytes comprise 77.6% of the total cell population. (c) CD20 expression is weak/dim and heterogenous. (d) Those cells show kappa light chain restriction. (e and f) CD10 and CD23 are negative (Lymph node).

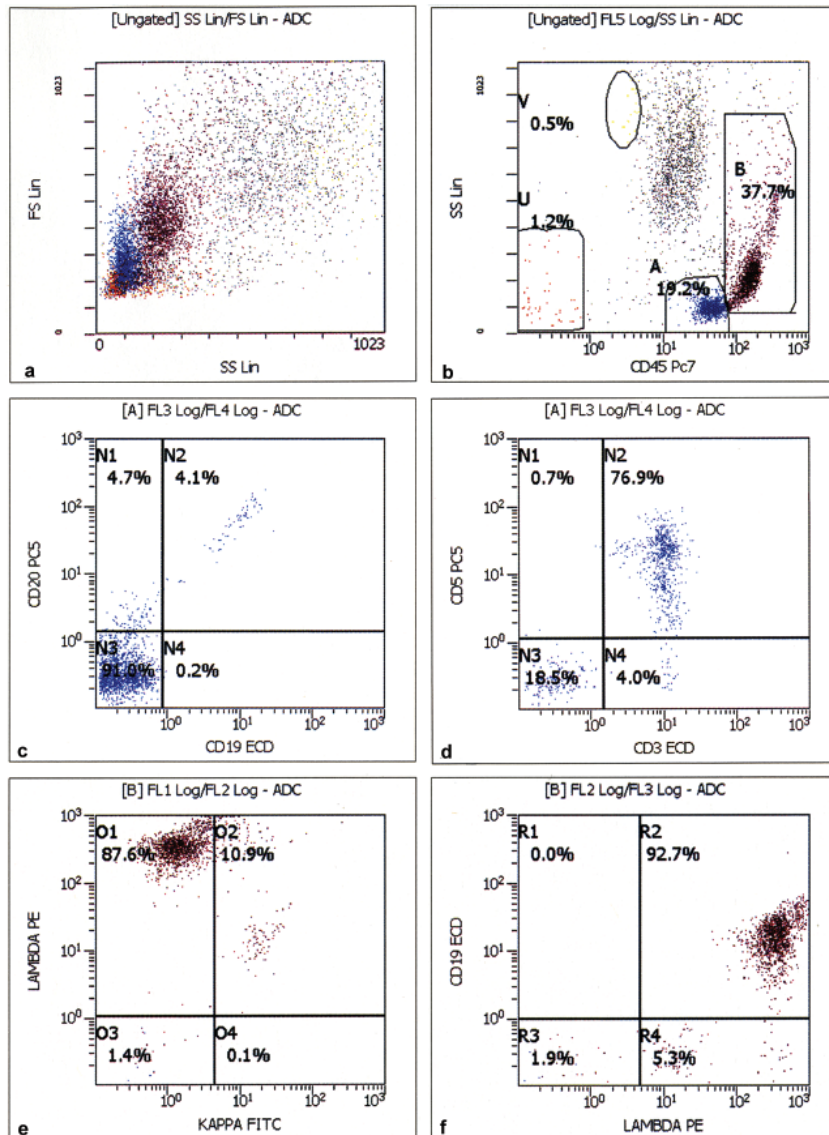


**Fig. 13-6B: Mantle cell lymphoma mimicking atypical CLL/SLL.** (g and h) The neoplastic B-cells co-expression of CD5 and CD19, but FMC7 is negative. The lymph node showed typical SLL morphology; however, FISH study showed a t(11;14) translocation. These findings are consistent with mantle cell lymphoma (Lymph node).

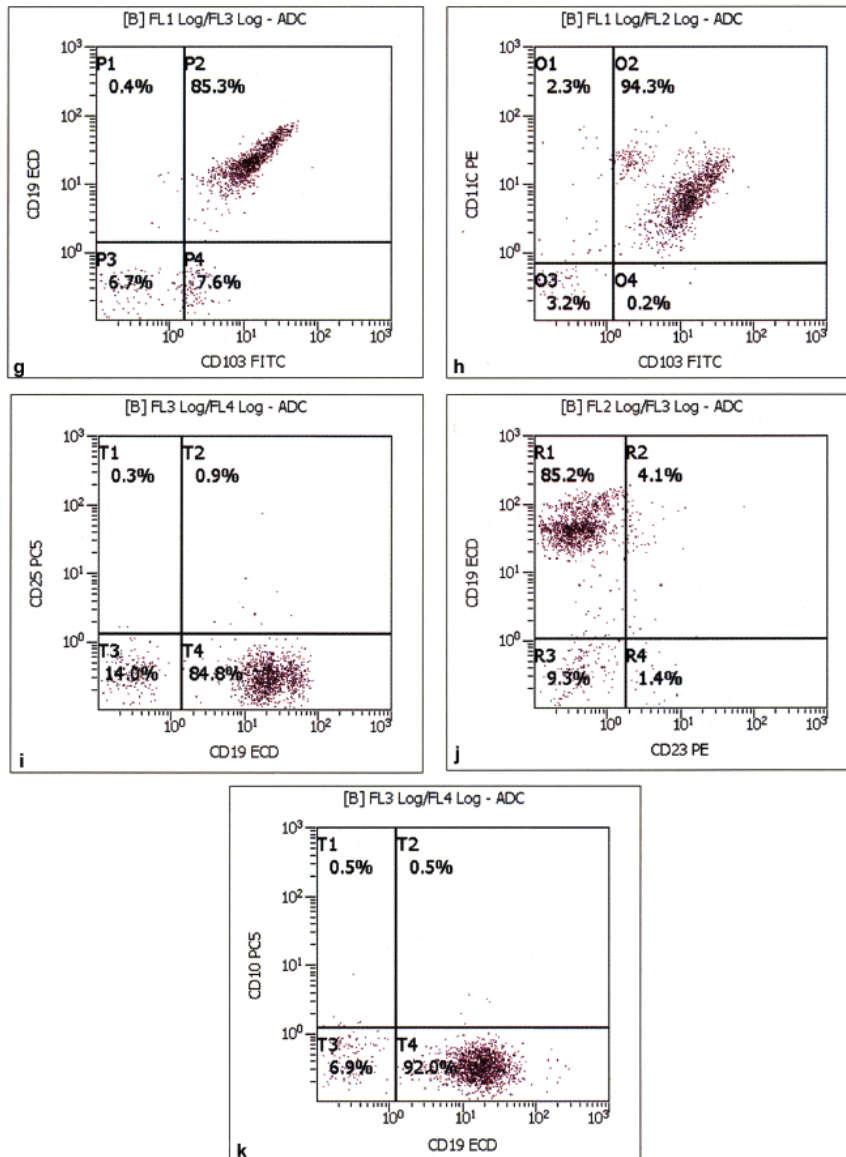




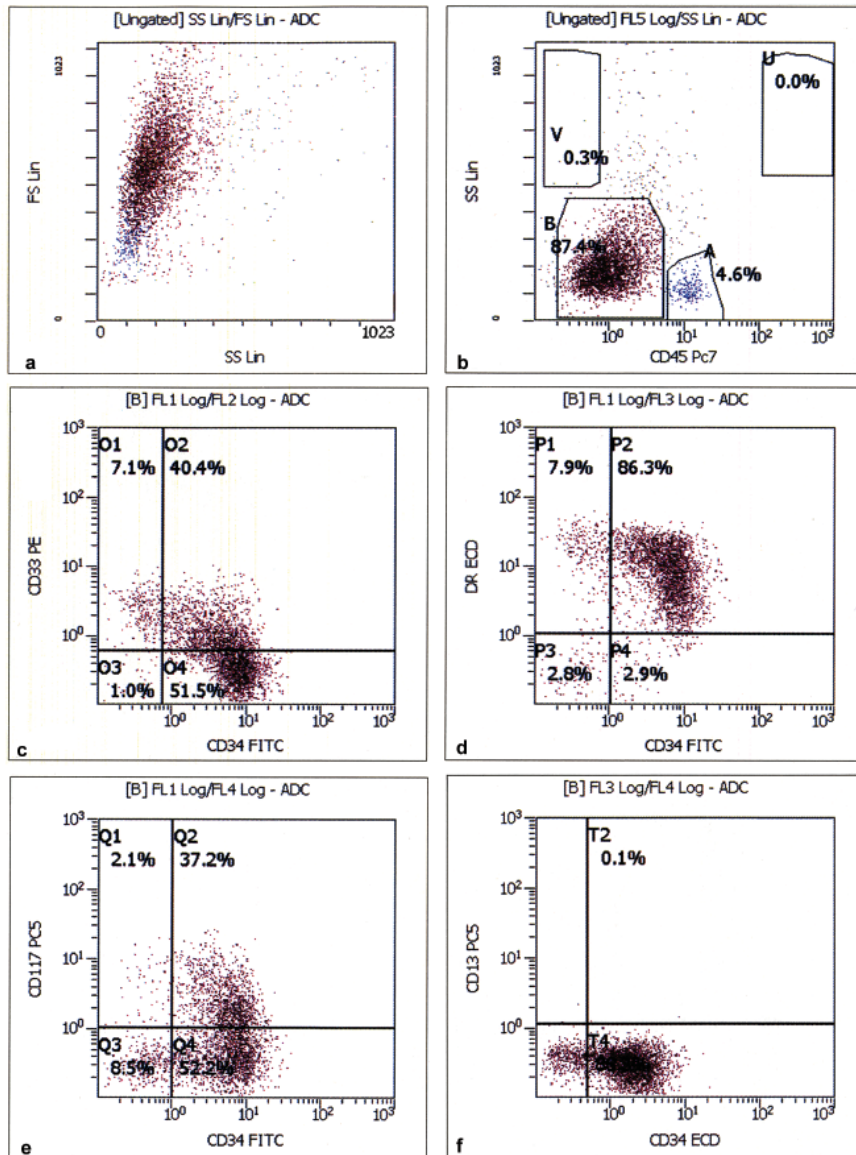
**Fig. 13-7: Follicular cell lymphoma.** (a and b) Lymphocytes comprise 92.4% of the total cell population. (c) Those B-cells are CD10 positive. (d and e) and Lambda light chain restriction. (f) There is no co-expression of CD5 and CD19 or CD19 and CD23 (data not shown) (Lymph node).



**Fig. 13-8A: Hairy cell leukemia (HCL).** (a and b) Two populations of cell: small (blue) and larger (dark brown). The larger cells show complex cytoplasmic contents and bright expression of CD45 (dark brown). (c and d) The small cells predominantly T-cells. (e and f) Those larger cells are B-cell with a Lambda light chain restriction (Peripheral blood).

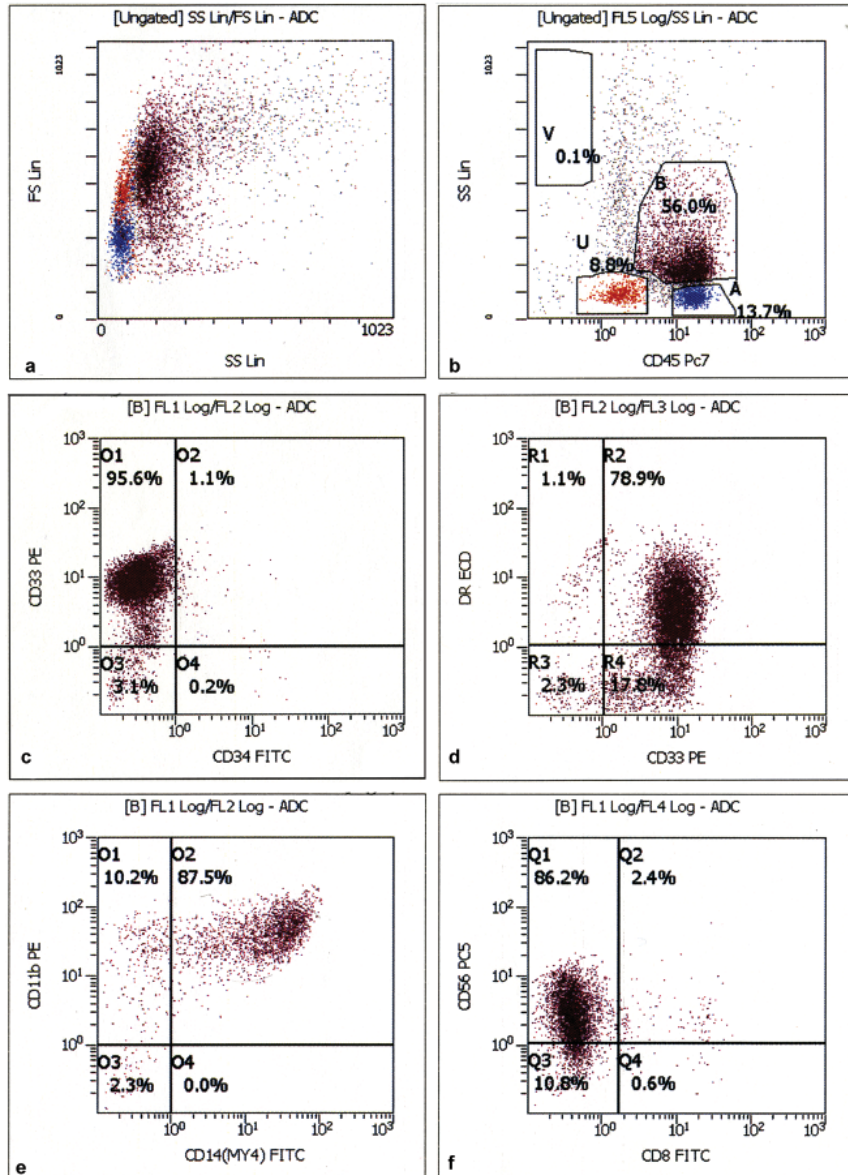


**Fig. 13-8B: Hairy cell leukemia (HCL).** (g and h) The characteristic hairy cell markers CD11c and CD103 are positive; (i) CD25 is negative in this case. (j and k) CD10 and CD23 are negative (Peripheral blood).

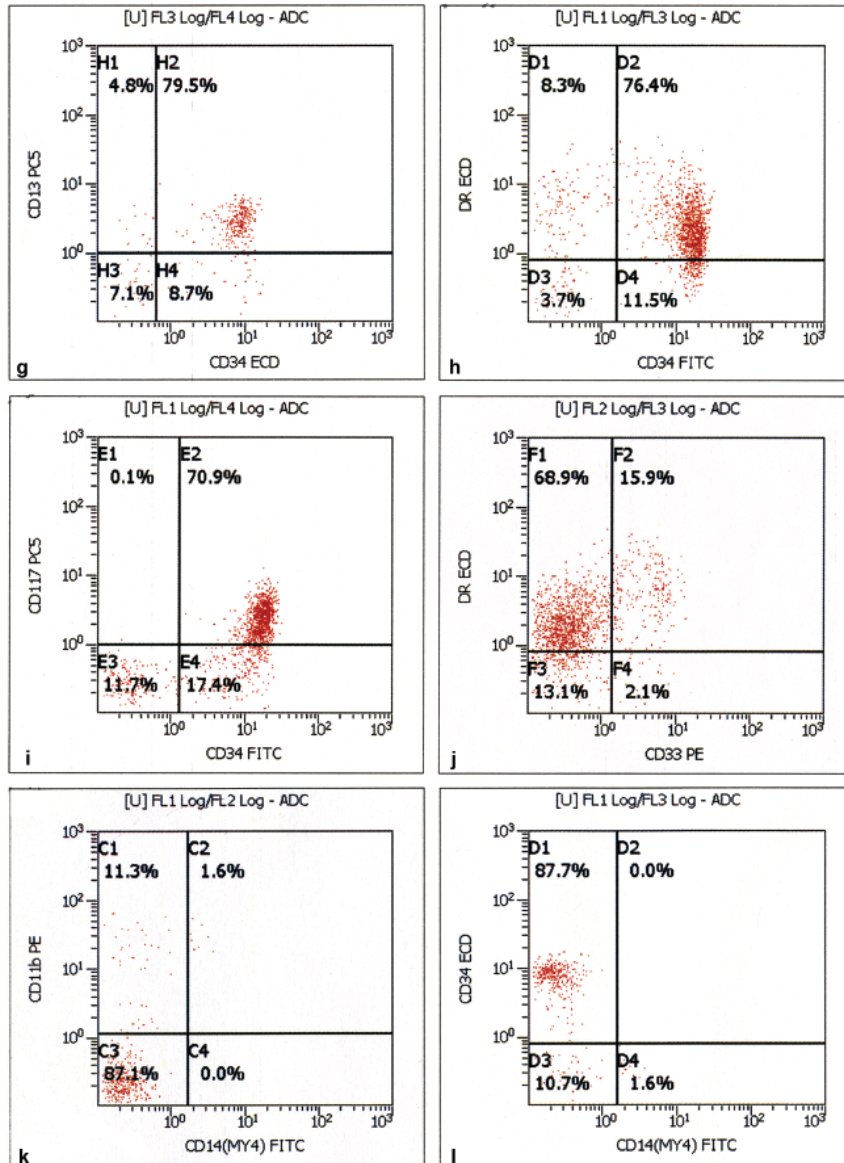


**Fig. 13-9: Acute myeloid leukemia (AML).** (a and b) A distinct population in the blast region with variable complexity of cytoplasmic contents and dim CD45 expression. (c, d and e) The blasts are positive for CD34, HLA-DR, CD33 (partial), and CD117 (partial). (f) CD13 is negative (Bone marrow).

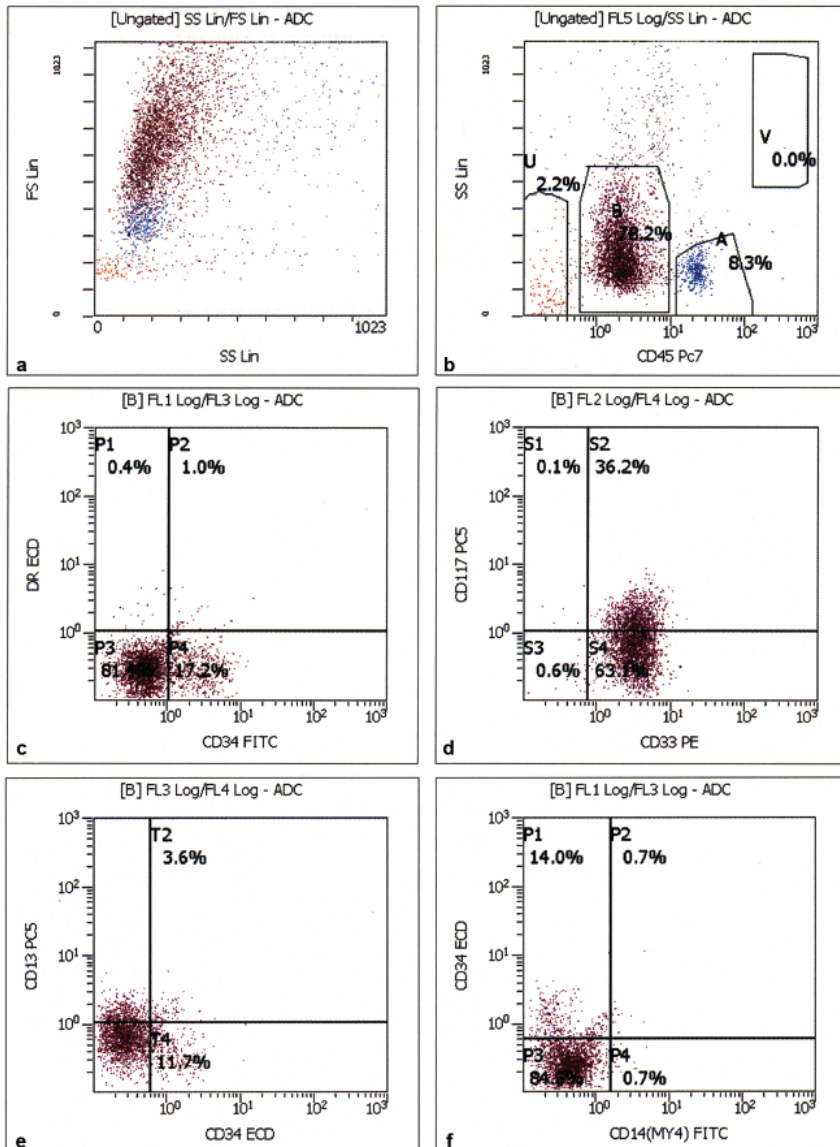




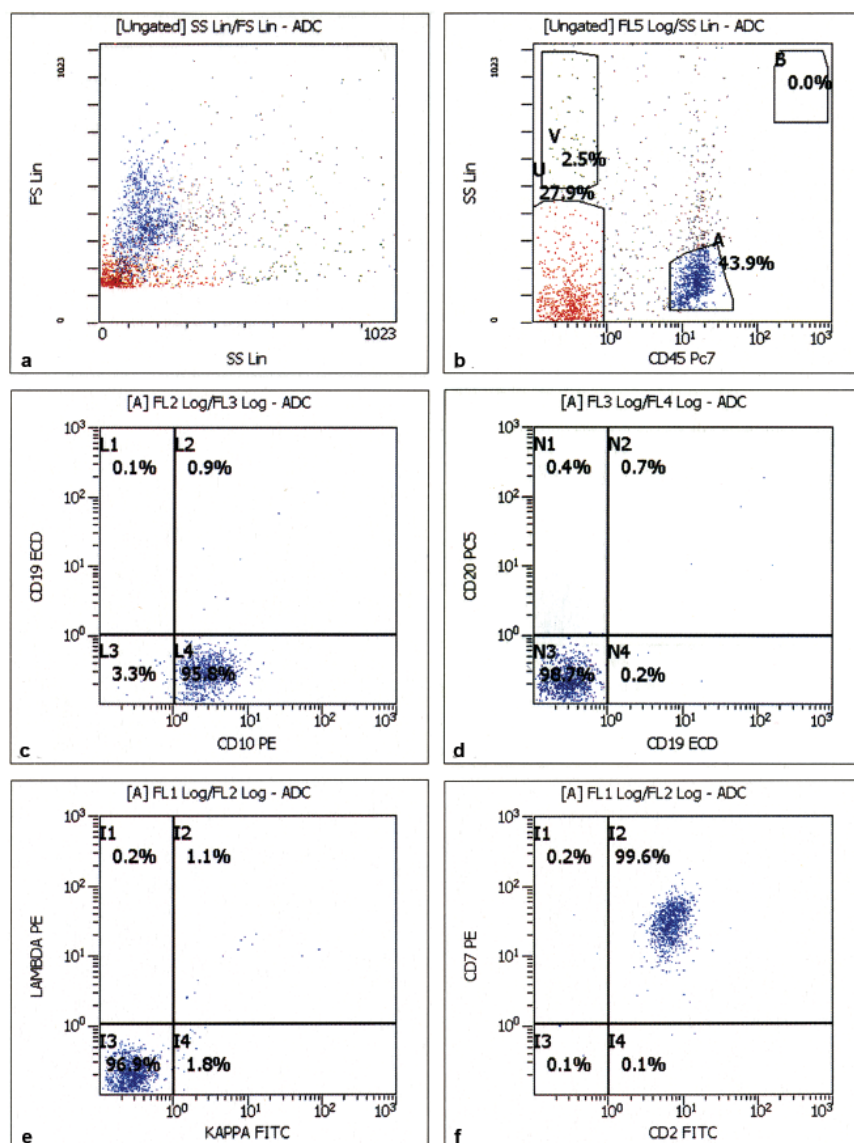
**Fig. 13-10A: Acute myelomonocytic leukemia (AMML).** (a and b) There are three distinct populations of cells: lymphocytes (blue), blasts (bright red) and a third population in the monocytic area (red-brown). (c and d) The cells in the monocytic region are CD33 and HLA-DR positive but CD34 negative. (e) Monocytic markers CD11b and CD14 are positive. (f) Aberrant expression of CD56 is also present (Bone marrow).



**Fig. 13-10B: Acute myelomonocytic leukemia (AMML).** (g, h and i) The cells in the blast region are CD13, CD34, CD117, and HLA-DR positive. (j) CD33 is negative (Bone marrow).

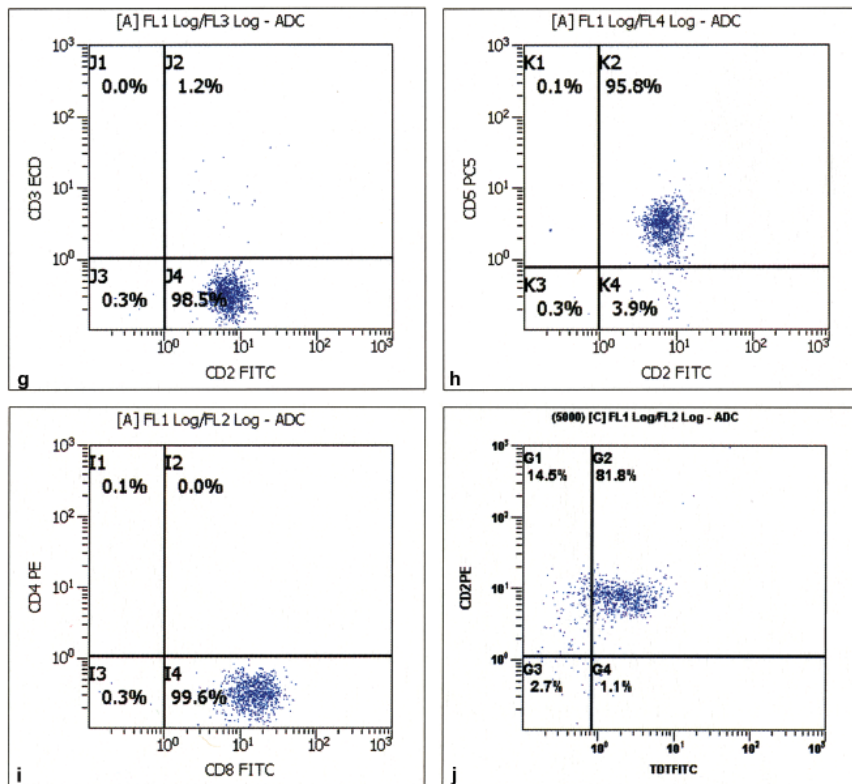


**Fig. 13-11: Acute promyelocytic leukemia (APL).** (a and b) A major population of blasts, with a wide range of side scatter is characteristic of APL. (c) The APL cells in the blast region are characteristically negative for CD34, and HLA-DR. (d and e) The APL cells are positive for CD33, CD13 (partial), and CD117 (partial). (f) Monocytic marker CD14 is negative (Bone marrow).

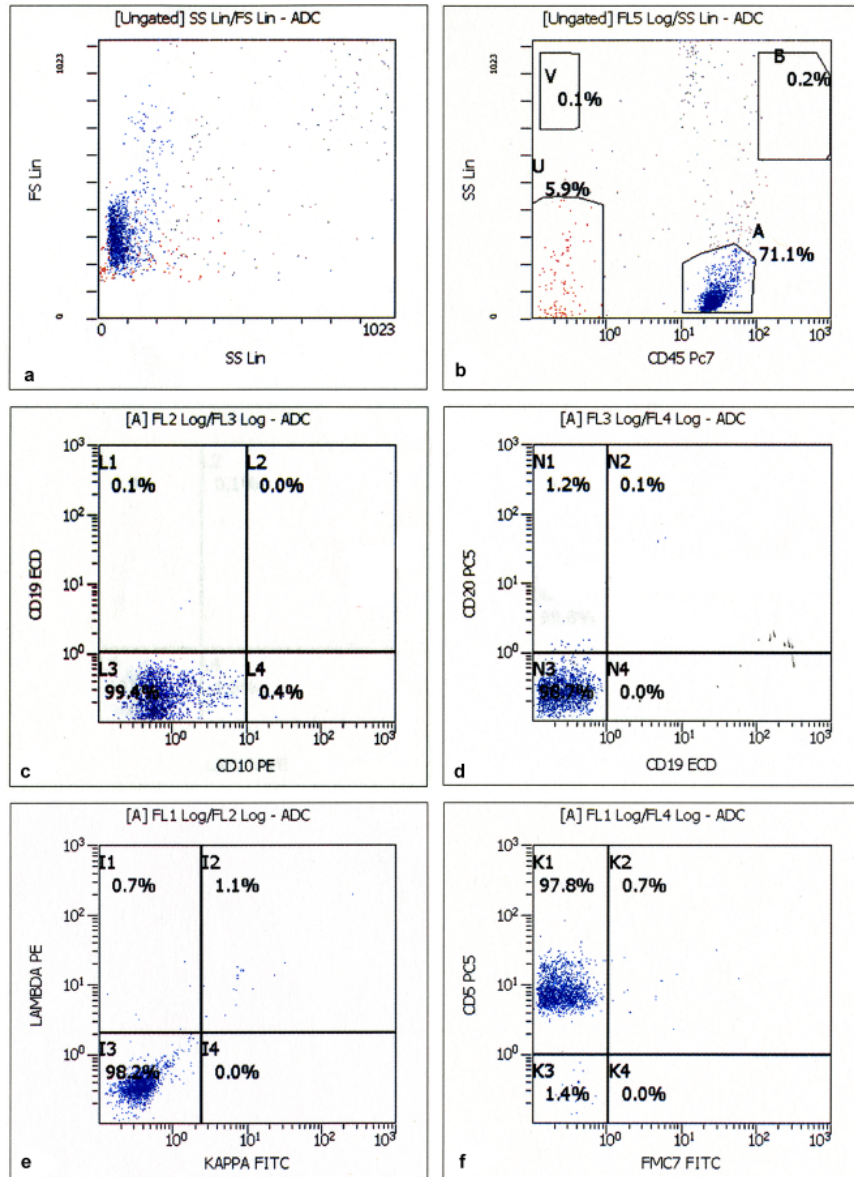


**Fig. 13-12A: Acute lymphoblastic lymphoma/leukemia (ALL).** (a and b) A population of lymphocytes comprises 43.9% of the total cell population. ALL Kappa may be located in the lymphoid region or blast region. (d and e) CD19, CD20, Kappa and Lambda light chain are negative. (c and f) CD2, CD7, and CD10 are positive (Mediastinal mass).

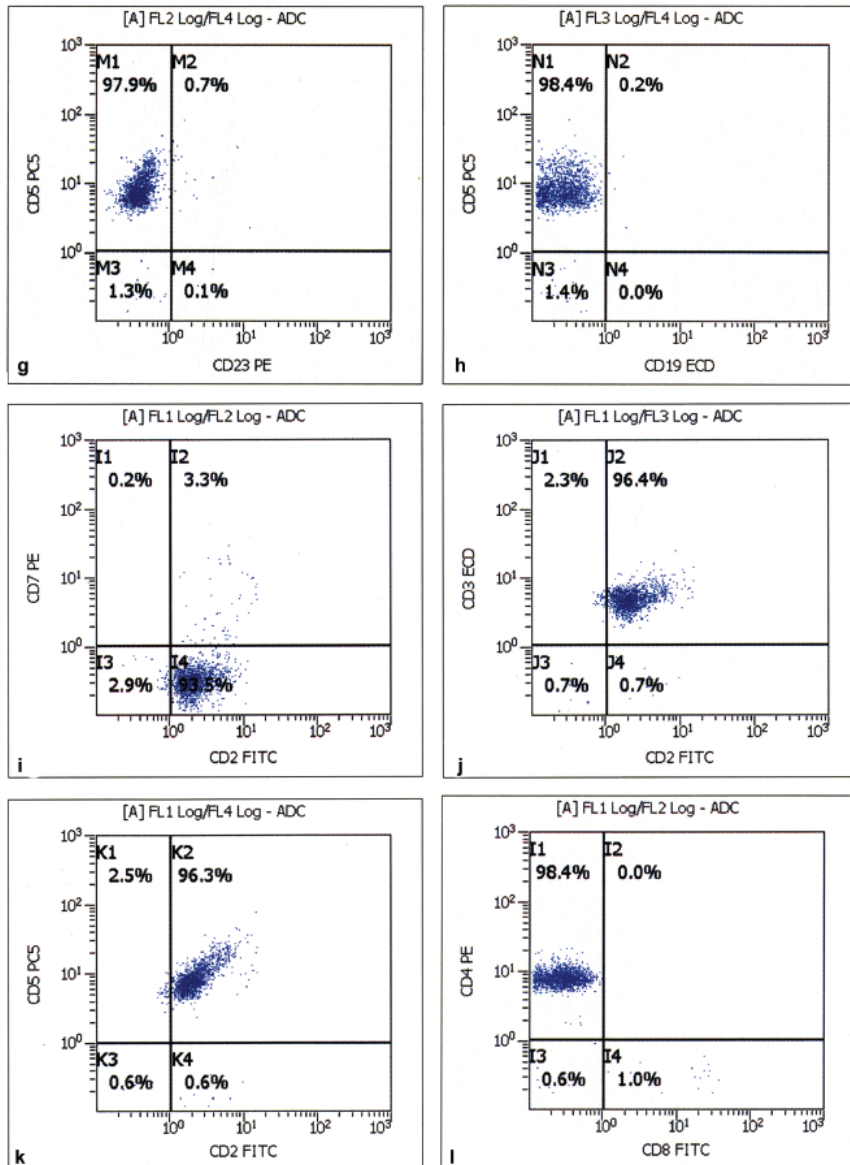




**Fig. 13-12B: Acute lymphoblastic lymphoma/leukemia (ALL).** (g and i) Surface CD3 and CD4 are negative. (h, i, and j) CD2, CD5, CD8, and TdT are positive (Mediastinal mass).



**Fig. 13-13A: Sézary syndrome.** (a and b) A population of lymphocytes comprises 71.1% of the total cell population. (c, d, and e) Those cells are negative for CD10, CD19, CD20, Kappa and Lambda light chains. (f) CD5 is positive but FMC7 is negative (Peripheral blood).



**Fig. 13-13B: Sézary syndrome.** (g, h, i, j, k and l). Those cells are positive for T-cell marker CD2, CD3, CD4, CD5 and negative for CD7, CD8, and CD23 (Peripheral blood).

A large, light purple histological section of skin tissue, likely a hematoxylin and eosin (H&E) stain, showing the epidermis and dermis. A red horizontal bar is overlaid across the middle of the image.

CHAPTER

14

# Molecular Pathology



### *Basic Concept and Terminology*

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**Alpha satellite DNA** is a tandemly repeated 171 base pair DNA that resides at the centromeres of chromosomes. Alpha satellite DNA can be used to identify chromosomes by fluorescence in situ hybridization (FISH).

**Autosomal dominant inheritance:** A single mutant allele on one chromosome (heterozygote) is sufficient to cause clinical disease. Equally affects males and females. Each offspring has a 50% chance of inheriting the mutant allele.

**Important autosomal dominant disorders:**

1. **Huntington's disease (HD)** is a disorder characterized by neurological degeneration. The HD gene IT15 is located on chromosome 4p16.3 that contains **CAG** repeat. Normally these repeats are ranged from 10 to 26 bases; however, in HD, those repeats are increased from 36 to 121 bases.
2. **Familial breast-ovarian cancer** is caused by a germline mutation in one of the breast cancer genes BRCA1 or BRCA2. These mutations affect DNA damage repair mechanism. Patient has an increased risk of GI, ovarian and breast cancers.
3. **Hereditary diffuse gastric carcinoma** is caused by mutations in the CDH1 gene, which affects cell-to-cell adhesion.
4. **Familial adenomatous polyposis** is caused by mutations in the APC gene, which controls transcription. Patient has an increased risk of GI cancers.
5. **Juvenile polyposis** is caused by mutations in the SMAD4/DPC4 genes that affect TGF $\beta$  signaling pathway. Patients have an increased risk of GI cancers.
6. **Cowden syndrome** is caused by mutations in the PTEN gene. Patient has an increased risk of GI, breast, endometrial, kidney, and thyroid cancers.
7. **Li-Fraumeni syndrome** is caused by mutations in the TP53 gene that affect cell apoptosis. Patient has an increased risk of GI cancers.
8. **Peutz-Jeghers syndrome** is caused by mutations in the LKB1/STK11 genes that affect serine/threonine kinase. Patient has an increased risk of GI cancers.
9. **Hereditary nonpolyposis colon cancer syndrome (HNPCC syndrome)** is caused by mutations in the MSH2 and MLH1 genes, which affect DNA mismatch repair mechanism. Patient has an increased risk of GI cancers.

10. **von Hippel-Lindau syndrome** is caused by mutations in the VHL gene. Patient may have hemangioblastoma in retina, kidney, spinal cord or cerebella, and an increased risk for renal angioma, renal cell carcinoma or pheochromocytoma.

**Autosomal recessive inheritance:** The alleles on both chromosomes are mutated (homozygote). If only one allele is affected (heterozygote), the person is a carrier. This inheritance affects males and females equally. Offspring of two carriers have a 25% chance of being affected, 50% chance of being carriers and 25% chance of being normal.

**Important autosomal recessive disorders:**

1. **Friedreich's ataxia** is a disorder characterized by preadolescent onset of symptoms, progressive cerebellar dysfunction, and hypoactive reflexes in the lower limbs. The Friedreich's ataxia gene **FRDA** is located on chromosome 9p13, which contains **GAA** repeats. Normally GAA repeats are less than 40 bases, in Friedreich's ataxia, GAA repeats are ranged from 66 to 1700 bases. Increased GAA repeats will affect frataxin synthesis. Decreased level of frataxin results in accumulation of iron and free radicals in mitochondria, and damages the mitochondria.
2. **Phenylketonuria (PKU)** is a disease caused by mutations in the phenylalanine hydroxylase gene (12q22-q24.1). Approximately 400 mutations are identified, six mutations account for 60% of all mutations in the Caucasians.
3. **Galactosemia** is a disease due to several enzymatic deficiencies that caused by mutations in the galactose-1-phosphate uridylyltransferase gene (9p13).
4. **Multiple adenomatous polyposis** is caused by mutations in the MYH gene that affect DNA damage repair. Patient has an increased risk of GI cancers.
5. **Cystic fibrosis** is a disease caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene (7q31.2). More than 1000 mutations in the CFTR gene have been reported. The most common mutation among Caucasian patients is a deletion of codon 508 (70%) in the CFTR gene.
6. **Hereditary hemochromatosis** is a disease caused by mutations in the HFE gene (6p).
  - C282Y mutation is found in approximately 83% of cases.
  - H63D mutation is found in approximately 10% of cases.

**Chromosome microdeletion or microduplication** is a deletion or duplication that involves only millions base of DNA, and is undetectable under conventional cytogenetic examination.

**Syndromes associate with microdeletion:**

1. Alagille syndrome (20p12)—abnormalities of heart, eye, skeleton, and kidney.
2. Angelman syndrome (15q11-13)—ataxic gait.
3.  $\alpha$ -thalassemia and mental retardation (16p13.3)— $\alpha$ -thalassemia (deletion of 16p  $\alpha$ -globin locus) and mental retardation.
4. DiGeorge syndrome (22q11)—abnormalities of third and fourth branchial arches.
5. Langer-Giedion syndrome (8q24.1)—sparse hair, bulbous nose and mental retardation.
6. Miller-Dieker syndrome (17p13)—dysmorphic facies and lissencephaly.
7. Prader-Willi syndrome (15q11-13)—obesity, hypogonadism and mental retardation.
8. Smith-Magenis syndrome (17p11.2)—brachycephaly, midface hypoplasia, and mental retardation.
9. William syndrome (7q11.23, elastin gene)—congenital heart disease (usually supravalvular aortic stenosis), facial dysmorphic, gregarious personality, premature aging, and mental retardation.
10. WAGR complex (11p13)—Wilms' tumor, aniridia, genitourinary disorders, and mental retardation.

**Syndromes associate with microduplication:**

1. Beckwith-Wiedemann (11p15)—macrosomia, macroglossia and omphalocele.
2. Charcot-Marie-Tooth syndrome type 1A (17p11.2)—progressive neuropathy.

■ **Complementary DNA (cDNA)** is a DNA synthesized from a mature mRNA template

■ **DNA** is a double-stranded polymer composed of nucleotides adenine (A), cytidine (C), guanine (G), and thymidine (T).

■ **DNA mismatch repair** is an evolutionarily conserved process that corrects mismatches generated during DNA replication and escape proofreading. Inactivation of DNA mismatch repair may result in developing cancer.

Muir-Torre syndrome (MTS) is characterized by adenomatous colonic polyp, increase risk of breast and genitourinary tract malignancies, skin

keratoacanthomas and sebaceous tumor. The genes affected are MLH1 and MSH2.

DNA mismatch repair genes (**MSH2** and **MLH1** genes account for 90% of the cases):

MSH2 (2p21)	MLH1 (3p21)
PMS1 (2p31)	PMS2 (7p22)
MSH6 (2p21)	MSH3 (5q14)

■ **Enhancer** is a sequence that is located on either the upstream or the downstream of RNA transcription initiation site that increases the rate of transcription.

■ **Epigenetics** is the study of heritable changes in genes expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. Epigenetics includes methylation of DNA, and modification of histone proteins.

■ **Exon** is a protein-coding or functional non-protein coding DNA sequence, which is transcribed to mRNA, rRNA or tRNA.

■ **Fusion gene** is a hybrid gene formed from two previously separate genes. It can occur as the result of a translocation or interstitial deletion. Fusion gene may be oncogene such as BCR-ABL.

■ **Intron** is a noncoding DNA sequence, which is located between exons. Introns are removed (spliced out) during transcription of mRNA. Alternative splicing introns can generate multiple proteins from a single gene.

■ **Inversion** is the breakage of a chromosome in at least two places, followed by rotation and rejoining of the involved segment.

■ **Imprinting** (Genomic imprinting) is an epigenetic process by which the male and female genomes are differently expressed. The imprinting mark on genes is either by DNA methylation or histone protein modification. The imprinting patterns are different according to the parental origin of the genes. Prader-Willi syndrome and Angelman syndrome are examples of imprinting disorders. In Prader-Willi syndrome, both 15q13 regions are from the father, whereas in Angelman syndrome both 15q13 regions are from the mother.

■ **Isochromosome** is a chromosome that has two copies of either the short or the long arm, fused at or near the centromere.



■ **Isoschizomers** are pairs of restriction enzymes that are specific to the same recognition DNA sequence.

■ **Messenger RNA (mRNA)** is a template that transfers genetic information from genes to ribosomes where the genetic sequence is used as a template for protein synthesis.

■ **Methylation of DNA** is the process that adds of a methyl group to a cytosine pyrimidine ring, or adenine purine ring. Methylation of the cytosine residue leads to decreased or inactivated of a gene expression.

Tumor suppressor genes that inactivated by aberrant methylation or hypermethylation include:

- APC
- BRCA1
- MLH1
- FHIT
- p14
- p15
- p16

■ **Methylation sensitive/insensitive restriction enzyme** is used to detect methylated region of DNA.

■ **Missense mutation** is a mutation leads to the formation of a new amino acid.

**Important diseases due to missense mutation:**

1. Sickle cell disease.
2. Hemoglobin C disease.
3. Factor V Leiden: A **single point mutation** in the factor V gene (A506G) affects active protein C cleave site resulting in unable to inactive factor V.
4. Prothrombin G20210A: A **single point mutation** in the 3' untranslated gene of prothrombin resulting in increased gene expression.

■ **Mitochondrial inheritance** is maternally inherited. A mutated mitochondrial DNA (MtDNA) can affect both male and female offsprings, but only females transmit the mutation.

**Mitochondrial DNA mutation related diseases:**

1. Leber's hereditary optical neuropathy is a disorder due to MtDNA mutations, and characterized by deterioration or loss of central vision.
2. Kearns-Sayre syndrome is a disorder due to MtDNA deletion or duplication, and characterized by progressive external ophthalmoplegia, and pigmentary retinopathy.

3. Myoclonic epilepsy with ragged red fibers is a disorder due to Mt t-RNA point mutation, and characterized by constellation of symptoms, and deafness.
4. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes are a disorder due to Mt tRNA point mutation.

■ **Nondisjunction** is the failure of chromosomes to separate during cell division, resulting in a cell containing too few or too many chromosomes (trisomy 21, Down's syndrome).

■ **Nonsense mutation** is a mutation, which leads to the formation of one of the STOP codons.

■ **Oncogenes** are the activated form of proto-oncogenes.

#### **Important oncogenes and their associated diseases:**

1. ABL (9q31.4): CML.
2. BCL2 (18q21): Follicular cell lymphoma and CLL/SLL.
3. CCND1 (11q13.3): Also known as Cyclin D1. Overexpression in mantle cell lymphoma, parathyroid adenoma, breast cancer, and plasma cell neoplasm.
4. ERBB2 (17q21.1): Also known as Her-2/neu, overexpression in breast, ovarian, and lung cancers.
5. RAS: RAS family includes a number of proteins that have structural and functional similarities.
  - H-RAS (11p15.5-p15.1): Breast, lung, kidney, bladder, and colon cancers
  - K-RAS (12p): Associated with lung cancer (predominantly adenocarcinoma), colon and other tumors.
6. C-MYC (8q14.13-q24.13): Burkitt's lymphoma, breast, lung, and ovarian cancers.
7. N-MYC (2p24.1) associated with neuroblastoma, over expression is associated with poor prognosis.
8. NF1 (17q11.2): Neurofibromatosis.
9. RET (10q11.2): Associated with MEN II syndrome, thyroid carcinoma, **Hirschsprung's disease**, and Hürthle cell adenoma.

■ **Penetrance** is the number of individuals with a specific genotype who express the corresponding phenotype.

■ **Promoter** is a sequence that is located on the upstream of a RNA transcription initiation site, which is involved in the recruitment of RNA polymerase II (TATA box is a common promoter).

- **Proto-oncogenes** is a normal gene that encodes protein, which involves cellular mitogenic signaling and growth control.
- **Pseudogene** is a gene derived from functional genes, but nonfunctional due to mutations in the gene.
- **Reading frame shift:** Any deletion or insertion of nucleotides that is not a multiple of three will lead to change of reading frame to a different amino acid sequence.
- **Restriction enzyme** is an enzyme that isolated from bacteria, it cuts DNA at specific sequence sites known as restriction sites.
- **Restriction fragment length polymorphism (RFLP):** Human genomic DNA is polymorphic; restriction enzyme digestion will generate different length of fragments between individuals. The pattern of fragments is analyzed via the Southern blot procedure. RFLP can be used for forensic identity, genome mapping, inheritance of genetic traits, and paternity testing.
- **Reverse transcription:** The usual direction of genetic information flow is from DNA to RNA to protein. Genetic information flow from RNA to cDNA to RNA to protein (under reverse transcriptase) is called reverse transcription. Such as HIV.
- **Ribosomal RNA (rRNA)** is the structure on which proteins are synthesized
- **RNA** is a single-stranded polymer composed of nucleotides adenine (A), cytidine (C), guanine (G), and uracil (U).
- **Robertsonian translocation** is the breakage and fusion of two acrocentric chromosomes near their centromeres with resulting loss of their short arms.
- **Short tandem repeat (STR)** is composed of multiple 2-6 base pairs length of repeats. STR analysis has been used in bone marrow transplant (compare the donor and recipient STR length) and forensic identity test.
- **Silent mutation** is a point mutation that does not result in change of amino acid.
- **Simple sequence repeat (SSR)** is a mono-, di-, or trinucleotides repeats. Important disease that related to repeat are fragile X syndrome (**CGG** repeat) and Huntington's disease (**CAG** repeat). These repeats may be used for paternity, forensic testing and bone marrow transplant monitoring.
- **Telomerase** is an enzyme that adds telomere sequence (TTAGGG) to the end of chromosomes, which stabilizes and maintains the telomeres length in a normal dividing cell. Telomerase is reactivated in most malignancies.

■ **Telomere** is multiple copies of repetitive sequence (TTAGGG) at the end of chromosomes. Telomeres will short a bit during cell division and chromosome duplication. It is related to normal aging process.

■ **Transcription** is the process of synthesizing messenger RNA (mRNA) from DNA. It includes:

1. Add a 7-methyl-guanosine cap at the 5' end to stabilize the mRNA against exonuclease (enzyme) degradation.
2. Remove introns.
3. Add a poly A tail at the 3' end.

■ **Transfer RNA (tRNA)** is the information adapter molecule during protein synthesis, tRNA decodes the information in DNA and direct interface between amino acid sequence of a protein and the information in DNA.

■ **Translation** is the process that genetic code in mRNA is read three nucleotides at a time to synthesize corresponding amino acid.

■ **Translocation** is the breakage of chromosomes followed by exchanging and rejoining between two chromosomes.

■ **Tumor suppressor gene** encodes proteins, which regulate normal cell proliferation. Mutations in these genes result in deregulation of the cell cycle. In general, loss of function of both alleles is needed (two hits) to develop tumors. RB1 (protein product **pRB**) and TP53 (protein product **p53**) are the two most critical genes related to the development of a wide range of tumors.

#### **Important tumor suppressor genes and their associated diseases:**

1. APC (5q21): Familial adenomatous polyposis.
2. DCC (18q21), MCC (5q21), APC (5q21): sporadic GI tumors.
3. RB1 (13q14): Inherited tumor of retinoblastoma, also related to sporadic tumors.
4. TP53 (17p13): Inherited tumor of Li-Fraumeni, also related to sporadic tumors (colon, brain, lymphoid/leukemia, lung, liver, astrocytoma, and other solid tumors).
5. WT1 (11p13): Wilms tumor.
6. WT2 (11p15): Inherited tumor of Wiedemann-Beckwith syndrome, renal rhabdoid tumors, and embryonal rhabdomyosarcoma.
7. NF1 (17q11): Neurofibromatosis I
8. NF2 (22q11): Neurofibromatosis II, ependymoma, meningioma.
9. VHL (3p25): von Hippel-Lindau and renal tumors.
10. MEN1 (11q23) multiple endocrine neoplasia type I.



11. NM23 (17q21), CDKN2 (9p21): Inherited tumor of melanoma, also related to sporadic tumors.

■ **Uniparental disomy:** Under normal circumstances, one member of each homologous pair of chromosomes is of maternal origin from the egg and the other is of paternal origin from the sperm. In uniparental disomy, both copies of a particular chromosome pair originate from the same parent. If uniparental disomy is caused by an error in the first meiotic division, both homologous chromosomes of that parent will be present in the gamete - a phenomenon called heterodisomy. If the disomy is caused by an error in the second meiotic division, two copies of the same chromosome will be present through the mechanism of rescue, duplication, and complementation - a phenomenon called isodisomy. Isodisomy may also occur as a postfertilization error.

■ **Uniparental heterodisomy:** See uniparental disomy.

■ **Uniparental isodisomy:** See uniparental disomy.

■ **Unstable trinucleotide repeat diseases** are due to the expansion of trinucleotide repeats beyond a critical point, results in disease (Fragile X syndrome, Huntington's disease).

■ **Variable number tandem repeats (VNTR)** is a class of satellite DNA, which has a specific sequence that repeats multiple times. Some loci are highly variable in terms of the number of repeats. This feature can be used for identity testing by Southern blot, or PCR.

■ **X-linked dominant inheritance** is an uncommon mode of inheritance. Affected females transmit the disease allele to 50% of their offspring. Affected males transmit the disease allele to all of their female offspring and none to their male offspring.

■ **X-linked recessive inheritance:** In this mode of inheritance, males are affected and females are heterozygous carrier. Heterozygous carriers transmit the disease allele to 50% of their male offspring, and 50% of their female offspring are heterozygous carriers.

Affected males transmit the disease allele to all of their female offsprings (carriers) and none to their male offspring.

1. Fragile X syndrome is an X-linked recessive disorder characterized by mental retardation. Fragile X syndrome gene FMR1 is located on chromosome Xq27.3. This syndrome is caused by the expansion of **CGG** repeat in the 5' untranslated region of FMR1 gene. In the normal population, the CGG repeat at FMR1 gene is 5–50 copies. CGG repeat

54–200 copies is classified as premutation, and CGG repeat > 200 copies is classified as full mutation (disease).

Fragile X syndrome is also related to the abnormal methylation of FMR1 gene.

## *Methods in Molecular Pathology*

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### **Specimen Collection**

1. **Peripheral blood and bone marrow:** Use EDTA or acid citrate dextrose, but not heparin. Heparin can inhibit PCR reaction.
2. **Solid tissue:** Fresh frozen or formalin fixation.

### **Sources of Nucleic Acid**

1. **DNA** can be obtained from fresh tissue (used for Southern blot and PCR), or formalin fixed paraffin embedded tissue (PCR).
2. **RNA** obtained from fresh tissue only.

### **Southern Blot**

**Southern blot** is a method for detecting a specific DNA sequence in DNA samples. Southern blotting combine transfer of enzyme digested and electrophoresis-separated DNA fragments to a Nylon membrane, and subsequent fragment detection by radioactive material labeled probe hybridization. The method is named after its inventor, the British biologist Edwin Southern. Western blot (for protein), and Northern blot (for RNA) employ similar principles.

#### **Applications:**

1. Detection of gene deletions or amplifications.
2. Detection of mutations that alter restriction enzyme cutting sites.
3. RFLP markers for linkage studies.
4. DNA methylation studies (example: abnormal methylation of the FRAXA gene in the patients with fragile X syndrome).

### **Polymerase Chain Reaction**

**Polymerase chain reaction (PCR)** is a method used widely in clinical and research setting to amplify a small amount DNA into a quantity that suitable for testing.

A major problem associated with PCR is contamination, Ethenol and bleach are used to clean workbench. The work area is used to prepare PCR reagent must be separated from the work area where the DNA sample is loaded into the machine.

The order of preparing PCR sample is first the negative control (reagent only, no DNA sample is added, also called water control), then the patient's sample, and finally the positive control (a positive DNA sample). If the negative and positive controls are not what they should be, the test is invalidated, and must be repeated.

1. Equipment and reagent include:
  - a. A thermal cycler for rapid heating and cooling of the specimen
  - b. Two primers, which anneal to the 5' and 3' template DNA
  - c. DNA polymerase (thermal stable)
  - d. Deoxyribonucleotide triphosphates (dNTP)
  - e. Reaction buffer.
2. Temperature cycling of PCR
  - a. 94°C (denatures the double stranded DNA to single strands)
  - b. 55°C (anneals the primers to the template DNA, this temperature may be different based on GC content of the primer)
  - c. 72°C (extends the primer using DNA polymerase Taq).

These steps are repeated for 20-40 cycles to produce a quantity of DNA suitable for testing.

## **Real Time PCR**

Thermal cycler allows for continuous monitoring of the production of PCR products as the reaction proceeds during each cycle. A fluorescent reporter at one end and a quencher of fluorescence at the opposite end of the probe are used for DNA amplification. The close proximity of the reporter to the quencher prevents detection of its fluorescence. During the DNA amplification, Taq polymerase breaks the reporter-quencher proximity and thus allows unquenched emission of fluorescence. The fluorescence is proportional increased with the PCR product, and can be detected and measured in the real-time PCR thermocycler.

## **DNA Sequencing**

The most common method for DNA sequence is chain termination. Target DNA is amplified (by PCR) and then mix one primer, dNTP and four different color fluorescent-labeled di-deoxynucleotide triphosphates (ddNTP) in one

reaction tube. When a fluorescent-labeled ddNTP is incorporated, it will prevent further extension, different length fragments ended with a special fluorescent color labeled ddNTP will be generated. The fragments are separated by gel electrophoresis, and read by a laser scanner. This will allow for the determination of the DNA sequence. The development of fluorescent-labeled ddNTP makes automated, high-throughput DNA sequencing easier.

### Protein Truncation Test

**Protein truncation test** is a method of identifying nonsense mutations. The presence of a premature truncation mutation is indicated by synthesis a short peptide. This test is used as a research tools for BRCA1, BRCA2 or DMD (Duchenne muscular dystrophy).

### Fluorescence In Situ Hybridization

**Fluorescence in situ hybridization (FISH)** is a method that use fluorescent-labeled probe to hybridize the target chromosomes.

### Detecting Methylation of DNA

Methylation-specific PCR or Southern blot can be used. PCR is the prefer method.

### Gene Microarray Technology (Gene Chips)

Thousands of fluorescent-labeled probes are attached to a solid support base, and act as hybridization “anchors” for the target nucleic acids. Microscopic imaging system and a computer is used for analysis of patterns and levels of genes expression to provide important diagnostic and prognostic information.

### Microsatellite Instability Assays

Microsatellite is repeated sequences of DNA. These repeated sequences are common and normal. Microsatellite is consist of a sequence of repeating units of 1-6 base pairs in length, may become unstable due to defects in the normal DNA repair process. Microsatellite instability is a key factor in several cancers including colorectal, endometrial, ovarian, and gastric carcinomas. PCR is the most commonly used test to detect the shortening or lengthening of these repeat units.



## ***Genetic Abnormalities and Molecular Basis of Diseases***

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### **Common Recurrent Genetic Abnormalities in Soft Tissue and Bone Tumors**

See Table 14-1

#### **Breast Tumors**

1. Secretory type ductal carcinoma: Rearrangement of t(12;15)(p13;q25) results in a ETV6-NTRK3 fusion gene.
2. Inherited syndrome with an increased risk of breast cancer:
  - a. BRCA1 syndrome (BRCA1. BRCA1 mutation is associated with breast medullary carcinoma)
  - b. BRCA2 syndrome (BRCA2)
  - c. Li-Fraumeni syndrome (TP53)
  - d. Cowden syndrome (PTEN)
  - e. Hereditary non-polyposis colorectal cancer (MLH1, MSH2, MSH6, MLH3, and PMS2)
  - f. Peutz-Jeghers syndrome (STK11)
  - g. Ataxia telangiectasia (ATM)Her2/neu overexpression is associated with a poor clinical outcome.

#### **CNS Tumors**

1. Astrocytoma with a 10q deletion (PTEN deletion) has a poor prognosis.
2. Oligodendroglioma with a co-deletion of 1p/19q has a good prognosis and is sensitive to chemotherapy.

#### **Gastrointestinal Cancers**

1. Inherited syndrome with an increased risk of gastrointestinal cancer.
2. Familial breast cancer is caused by BRCA1 and BRCA2 gene mutations that affect DNA damage repair.
3. Hereditary diffuse gastric carcinoma is caused by CDH1 gene mutations that affect cell-to-cell adhesion.
4. Familial adenomatous polyposis is caused by APC gene mutations that affect transcription.

**TABLE  
14-1****Genetic abnormalities in bone and soft tissue tumors**

Aggressive angiomyxoma	12q15	HMGA2			
Aneurysmal bone cyst	17p13.2 16q22	USP6 CDH11	45-65% 6-25%		
Alveolar rhabdomyosarcoma	<b>t(2;13)(q35;q14)</b> t(1;13)(p36;q14) t(x;2)(q13;q35) t(2;2)(q35;p23) t(2;8)(q35;q13)	PAX3-FOXO1 PAX7-FOXO1 PAX3-FOXO4 PAX3-NCOA1 PAX3-NCOA2	72% 9%		<b>Worse prognosis</b>
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12) t(2;22)(q33;q12) t(12;16)(q13;p11)	EWSR1-ATF1 EWSR1-CREB1 FUS-ATF1			
Alveolar soft part sarcoma	<b>t(X;17)(p11;q25)</b> t(2;22)(q33;q12)	ASPSCR-TFE3 EWSR1-CREB1	>99%		
Clear cell sarcoma of soft parts	<b>t(12;22)(q13;q12)</b>	EWSR1-ATF1	>90%		
Congenital fibrosarcoma (infantile fibrosarcoma)	<b>t(12;15)(p13;q25)</b>	ETV6-NTRK3	>99%		
Desmoplastic small round cell tumor	<b>t(11;22)(p13;q12)</b>	EWSR1-WT1	>99%		
Dermatofibrosarcoma protuberans (DFSP)	<b>t(17;22)(q22;q13)</b>	COL1A1-PDGFB	>95%		
Giant cell fibroblastoma (juvenile form of DFSP)	<b>t(17;22)(q22;q13)</b>	COL1A1-PDGFB	>95%		
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21) t(3;9)(q12;q22)	EWSR1-NR4A3 TAF15- NR4A3 TCF12- NR4A3 TFG- NR4A3	75% 25%		Aggressive than the skeletal counterpart
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZF1-JJAZ1			
Ewing's sarcoma and peripheral neuroectodermal tumor (ES/PNET)	<b>t(11;22)(q24;q12)</b> <b>t(21;22)(q22;q12)</b> t(7;22)(p22;q12), t(17;22)(q12;q12) t(2;22)(q33;q12)	EWS-FLI1 EWS-ERG EWS-ETV1 EWS-E1AF EWS-FEV	95% 5% <1% <1% <1%		<b>Type 1</b> fusion of EWS to FLI1 exon 6 (better survival) <b>Type 2</b> fusion of EWS to FLI1 exon 5
Inflammatory myofibroblastic tumor	2p23 rearrangement	ALK fusion			ALK+
Lipoma	12q13-15 6p21-22 13q	HMGA2 HMGA2	<40% <10% <10%		
Low-grade fibromyxoid sarcoma	t(7;16)(q34;p11) t(11;16)(p13;p11)	FUS-CREB3L2 FUS-CREB3L1			
Malignant tenosynovial giant cell tumor	t(1;2)(p13;q37)	CSF1-COL6A3			Subset without t(1;2) has CSF1 overexpression
Myoepithelial tumor of soft tissue	t(1;22)(q23;q12) t(19;22)(q13;q12) t(6;22)(p21;q12) 16p11.2 rearrangement	EWSR1-PBX1 EWSR1-ZNF444 EWSR1-POU5F1			
Myxoid liposarcoma	<b>t(12;16)(q13;p11)</b> t(12;22)(q13;q12)	TLS-CHOP EWS-CHOP	>95% <6%		
Solitary fibrous tumor	12q13-15				
Synovial sarcoma	<b>t(X;18)(p11.23;q11)</b>  t(X;20)(p11.2;q13.3)	SS18-SSX1 SS18-SSX2 SS18-SSX4 SS18L1-SSX1	65% 35% <1%		Biphasic Monomorphic
Schwannoma	22q12 loss	NF2 loss			
Well differentiated liposarcoma	<b>12q14-15</b>	MDM2, CDK2, HMGA2, SAS	>95%		FISH for MDM2 and CDK2 is sensitive and specific

5. Multiple adenomatous polyposis is caused by MYH gene mutations that affect DNA repair.
6. Juvenile polyposis is caused by SMAD4/DPC4 gene mutations that affect TGF $\beta$  signaling pathway.
7. Cowden is caused by PTEN gene mutations that affect tyrosine phosphatase activity.
8. Li-Fraumeni is caused by TP53 gene mutations that affect cell apoptosis.
9. Peutz-Jeghers is caused by LKB1/STK11 gene mutations that affect serine/threonine kinase activity.
10. Hereditary nonpolyposis colon cancer is caused by MSH2 and MLH1 gene mutations that affect DNA mismatch repair.
11. Sporadic colon cancer: Cancer development includes stepwise acquisition of mutations, epigenetic changes, and alteration of gene expression. BRAF mutations are present in 40-50% of sporadic colon cancers and is **absent** in Lynch syndrome. The main molecular pathways include:
  - a. Chromosomal instabilities pathway
  - b. Microsatellite instabilities pathway
  - c. CpG island methylator pathway (wide spread CpG methylation in neoplasms)

The dominant genetic abnormality of GI cancers is inactivation of tumor suppressor genes (APC, p53, DCC, SMAD2, and SMAD4).
12. Hepatoblastoma: May involve abnormal activation of Wnt/ $\beta$ -catenin signaling pathway.
13. Hepatocellular carcinoma: Multiple genetic alterations may involve abnormal activation of Wnt/ $\beta$ -catenin signaling pathway.

The presence of KRAS mutations is associated with poor response to anti-EGFR therapy, worse prognosis and poor survival of patients with metastatic disease. The presence of BRAF mutations is mutually exclusive with KRAS mutations, and is associated with poor response to anti-EGFR therapy.

## Gynecologic Tumors

1. Cervical cancer and HPV infection.

HPV oncogenic ability is related to viral protein E5, E6 and E7

  - a. E5: acts on several membrane receptors that stimulate cell division
  - b. E6: binds p53 resulting in degradation of p53.

- c. E7: binds pRB resulting in degradation of pBR.  
 High-risk HPV type: **16, 18**, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.  
 Low-risk HPV type: **6, 11**, 42, 43, and 44.
2. Endometrial stromal tumor: t(7;17)(p15;q21) translocation results in JAZF1-JJAZ1 fusion gene (approximately 43% of cases).
3. Benign endometrial polyp and leiomyoma: Cytogenetic abnormalities are heterogeneous, rearrangement of 12q14-15 and 6p21 are common.
4. Mucinous ovarian tumor: KRAS mutation.
5. Complete mole: Fertilization of an empty ovum by a single sperm with subsequent duplication or by two sperm, P57 is negative (paternally imprinted).  
 46, XX (about 85%)  
 46, XY (about 15%)
7. Partial mole: Fertilization of a normal ovum by two sperm, P57 is positive.  
 69, XXY (about 70%)  
 69, XXX (about 27%)  
 69, XYY (about 3%)

## Lung Tumors

Multiple, complex genetic abnormalities and epigenetic changes, or tumor suppressor genes and oncogenes mutations, or promotor hypermethylation are commonly seen in lung tumors.

1. KRAS, EGFR and Her2/neu mutations are commonly associated with lung adenocarcinoma.
  - a. The presence of an EGFR mutation, EGFR amplification and protein overexpression is associated with significant, favorable response to EGFR tyrosine kinase inhibitor therapy, and longer survival.
  - b. The presence of KRAS mutations is associated with smoking, male gender, poorly differentiated tumors, and poor response to EGFR tyrosine kinase inhibitor therapy.
2. Pulmonary inflammatory myofibroblastic tumor (IMT): Tumor may contain rearrangement of 2p23 and ALK gene, this result in two fusion genes TPM4-ALK and TPM3-ALK. Immunohistochemical staining for ALK is positive.

## Renal Tumors

1. Clear cell type renal cell carcinoma (RCC): -3p.



2. Type 1 papillary RCC: +7, +17, -Y.
3. RCC with Xp11.2 (TFE3 fusion gene): Rare, primarily affect children and young adults.
4. Chromophobe RCC: Multiple abnormalities of chromosome 1, 2, 6, 10, 13, 16, 21, and Y.
5. Congenital mesoblastic nephroma (a low-grade fibroblastic sarcoma): Rearrangement of t(12;15)(p13;q25) results in an ETV6-NTRK3 fusion gene.  
Other tumors may contain ETV6-NTRK3 fusion gene include:
  - a. Infantile fibrosarcoma
  - b. Secretory carcinoma of the breast
  - c. Acute myeloid leukemia (rare).
6. Wilms' tumor (nephroblastoma): Abnormal expression of WT1 (11p13) and WT2 (11p15). The majority of anaplastic variant Wilms' tumor contains TP53 mutations.

## Salivary Gland Tumors

1. Pleomorphic adenoma
  - a. 40% of cases contain PLAG1 (8q21) rearrangement.
  - b. 8% of cases contain HMGA2 (12q15) rearrangement (may also present in carcinoma ex pleomorphic adenoma).
  - c. Other structural abnormalities.
2. Mucoepidermoid tumor: Rearrangement of t(11;19)(q21;p13) results in a MECT1-MAML2 fusion gene (27-63% of the cases).
3. Warthin's tumor: May contain rearrangement of t(11;19)(q21;p13).

## Testicular Germ Cell Tumors

i(12p) present in 50-70% of cases.

## Thyroid Tumors

1. Conventional papillary thyroid carcinoma is associated with the following gene mutation:
  - RET (10q11.2)
  - NTRK (1q22)
  - BRAF (7q34), a point mutation (V599E) correlates with advance disease at the presentation.

2. Follicular thyroid carcinoma: t(2;3)(q13;p25) translocation produces a PAX8-PPAR $\gamma$ 1 fusion gene, which is not present in papillary carcinoma (include follicular variant), anaplastic thyroid carcinoma, Hürthle cell carcinoma, or nodular hyperplasia. This fusion gene may present in follicular adenoma.
3. Medullary thyroid carcinoma: associated with RET mutations.

### **Hematopoietic Neoplasms may Contain JAK2 Mutation**

1. Polycythemia vera (> 95%)
2. Primary myelofibrosis (50%)
3. Essential thrombocythemia (40-50%)
4. Chronic myelomonocytic leukemia (13%, in one published study)
5. Chronic eosinophilic leukemia (rare)
6. Mastocytosis (rare)
7. ALL (rare)
8. Down syndrome associated leukemias (rare, JAK3 mutation is common).

### **Hematopoietic Neoplasms with an Increased Tyrosine Kinase Activity**

1. Chronic myelogenous leukemia.
2. Myeloid and lymphoid neoplasms with eosinophilia and PDGFA, PDGFB or FGFR1 rearrangement.
3. Mastocytosis with c-KIT mutation (also present in GIST).
4. AML with FLT3 mutation.



# **Questions and Answers**

### *Questions*

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1. Which of the following is correct, regarding Burst forming units (BFU<sub>E</sub>), colony-forming units (CFU<sub>E</sub>) and hematopoietic progenitor cells?
  - A. BFU<sub>E</sub> is the early progenitor cell committed to erythrocyte differentiation
  - B. CFU<sub>E</sub> is the early ancestor of the BFU<sub>E</sub>
  - C. BFU<sub>E</sub> has an unlimited capacity of proliferation and gives rise to granulocyte colonies
  - D. BFU<sub>E</sub> is sensitive to erythropoietin
  - E. IL3 has no effect on pluripotent and early progenitor cell development
2. Which of the following statements regarding IL-3 is correct?
  - A. Located on chromosome 5
  - B. Stimulates hematopoietic stem cells
  - C. Stimulates mast cells
  - D. Stimulates T cell subsets
  - E. All of the above
3. For patients who on Coumadin therapy, the INR is calculated as:
  - A.  $\text{INR} = (\text{Patient's PT} \times \text{mean PT}) \times \text{ISI}$
  - B.  $\text{INR} = (\text{Patient's PT} \times \text{mean PT})^{\text{ISI}}$
  - C.  $\text{INR} = (\text{Patient's PT} \times \text{mean PT}) / \text{ISI}$
  - D.  $\text{INR} = (\text{Patient's PT} / \text{mean PT})^{\text{ISI}}$
  - E.  $\text{INR} = (\text{Patient's PT} / \text{mean PT}) \times \text{ISI}$
4. The finding of a falsely elevated MCV in an anemic patient is most likely related to:
  - A. Vitamin B<sub>12</sub> deficiency
  - B. Folate acid deficiency
  - C. Cold hemagglutinin disease
  - D. Thalassemia
  - E. Iron deficiency
5. A very high MCHC suggests:
  - A. Thalassemia
  - B. Iron deficiency anemia
  - C. Spherocytosis
  - D. Chronic disease related anemia
  - E. Chronic blood loss



6. The patient's Hct is 45, absolute reticulocyte count is 3%, what is the reticulocyte index?
  - A. 1
  - B. 2
  - C. 3
  - D. 4
  - E. 5
7. Which of the following would NOT be affected by *in vitro* hemolysis?
  - A. RBC
  - B. MCV
  - C. MCH
  - D. MCHC
  - E. Hct
8. Which of the following is NOT a component of eosinophil granules?
  - A. Acid phosphatase
  - B. Eosinophil peroxidase
  - C. Acid hydrolases
  - D. Major basic protein
  - E. Alkaline phosphatase
9. Which of the following statement is correct regarding mast cells and basophils?
  - A. Basophils have mitotic potential
  - B. Mast cells have segmented nuclei
  - C. Mast cells contain myeloperoxidase
  - D. Mast cell granules contain heparin
  - E. Mast cells are normally found in peripheral blood
10. Erythropoietin (EPO):
  - A. Predominantly produced in the bone marrow
  - B. Produces more RBC by inducing proliferation of reticulocytes
  - C. Decreased in erythroid hyperplasia
  - D. Increased in aplastic anemia
  - E. Increased after transfusion
11. In a spun tube of blood, what is the correct sequence of layers (from top to bottom)?
  - A. Platelets, neutrophils, lymphocytes, monocytes, RBCs
  - B. Neutrophils, lymphocytes, monocytes, platelets, RBCs
  - C. Neutrophils, platelets, lymphocytes, monocytes, RBCs
  - D. Neutrophils, lymphocytes, platelets, monocytes, RBCs
  - E. Neutrophils, lymphocytes, monocytes, platelets, RBCs
12. The last myeloid cell capable of division is:
  - A. Myeloblast
  - B. Promyelocyte
  - C. Myelocytes
  - D. Metamyelocytes
  - E. Bands

- 13. The last erythroid cell capable of division is:**
- A. Pronormoblasts
  - B. Basophilic normoblast
  - C. Polychromatophilic normoblast
  - D. Orthochromic normoblasts
  - E. Polychromatic erythrocytes
- 14. Which of the following distribution regions of the body do neutrophils spend the least amount of time?**
- A. Bone marrow storage pool
  - B. Circulating pool
  - C. Tissue pool
  - D. Marginated pool
- 15. Where may neutrophils be found after they leave the bone marrow:**
- A. Located in peripheral blood for less than 24 hours, then marginate and enter the tissue
  - B. Located in peripheral blood for 48 hours, then marginate and enter the tissue
  - C. Located in peripheral blood for 72 hours, then marginate and enter the tissue
  - D. Located in peripheral blood for 48 hours, then marginate and enter the tissue then re-enter into peripheral blood
  - E. None of the above
- 16. Which of the following statements regarding interdigitating reticulum cells is correct?**
- A. Antigen presenting cells migrant from the skin
  - B. They are CD1a and S-100 positive
  - C. Phagocytosis is their main function
  - D. They are usually CD21negative
  - E. Interdigitating sarcoma is associated with Castleman's disease
- 17. Which factors cause increased proliferation of eosinophils?**
- A. IL-1
  - B. IL-5
  - C. Interferon gamma
  - D. Tumor necrosis factor
  - E. All of the above
- 18. Which of the following is correct regarding the function and significance of major basic protein in eosinophils?**
- A. Toxic to parasites
  - B. Neutralize heparin
  - C. Induce histamine release from basophils
  - D. Contain the crystalline core of granules
  - E. All of the above

**19. Plasma cells are:**

- A. Surface Ig negative and cytoplasmic Ig positive
- B. Most HLA-DR negative
- C. CD38 negative
- D. FMC-7 negative
- E. None of the above

**20. Which of the following will NOT stain reticulocytes?**

- A. New methylene blue
- B. Brilliant cresyl violet
- C. Pyronin Y
- D. Acridine orange
- E. Congo red

**21. The specific marker for early lineage B cells is:**

- A. CD10
- B. TdT
- C. CD34
- D. CD22
- E. CD20

**22. The immunophenotype of the small lymphocytes in the cortical area of a thymoma is:**

- A. TdT negative
- B. Co-expression of CD4/CD8
- C. Usually CD4-, CD8+
- D. Usually CD4+, CD8-
- E. Usually CD4-, CD8-

**23. The earliest T-cell marker to appear on the T-cell surface is:**

- A. CD2
- B. CD3
- C. CD4
- D. CD5
- E. CD7

**24. T-cell alpha and delta receptor gene is located at:**

- A. 14q11
- B. 7q35
- C. 7p14-15
- D. 14q32
- E. 8q24

**25. Which of the following statements regarding Ig heavy and light chain gene rearrangements is correct?**

- A. Kappa light chain is rearranged first then heavy chain of a joining segment to form DJ fusion
- B. Lambda light chain is rearranged first then heavy chain of a joining segment to form DJ fusion
- C. Heavy chain and light chain gene rearrangement only occur in B-cell malignancies
- D. Heavy chain of a joining segment to form DJ fusion, then lambda light chain rearrange if not successful the m kappa light chain rearrange
- E. heavy chain joint of a joining segment to form DJ fusion, then kappa light chain is rearranged if not successful, then lambda light chain rearranged

**26. The order of Ig heavy and light chain rearrangement is:**

- A. V-D-J, cytoplasmic mu, kappa and lambda
- B. V-D-J, cytoplasmic alpha, kappa and lambda
- C. Cytoplasmic delta, V-D-J, kappa and lambda
- D. Cytoplasmic gamma, V-D-J, kappa and lambda
- E. V-D-J, kappa and lambda, cytoplasmic mu

**27. Which description of T-cell gene rearrangement is correct?**

- A. The order of rearrangement is gamma, delta, beta and alpha
- B. The order of rearrangement is alpha, beta, gamma and delta
- C. Gamma, delta receptor present on 95% of the circulation T-cells
- D. TCR genes do not undergo V(D)J rearrangement
- E. Alpha and Beta chain genes are both located on chromosome 14q11.2

**28. Which of the following conditions is associated with only T-cell deficiency?**

- A. DiGeorge syndrome
- B. Common Variable Immunodeficiency syndromes (CVTD)
- C. Wiskott-Aldrich syndrome
- D. Adenosine deaminase deficiency
- E. Ataxia telangiectasia

**29. Which of the following disorders DOES NOT result in immune deficiency?**

- A. May-Hegglin anomaly
- B. Chediak-Higashi syndrome
- C. Chronic granulomatous disease
- D. Alder-Reilly anomaly
- E. Kostmann's disease



**30. Where is Gower Hb produced?**

- A. Spleen
- B. Liver
- C. Bone marrow
- D. Yolk sac
- E. Thymus

**31. Which condition will NOT affect RBC cytoplasmic maturation?**

- A. Chronic blood loss
- B. Aplastic anemia
- C. Thalassemia
- D. A and B
- E. A, B and C

**32. Which of the following infections may cause of marked lymphocytosis?**

- A. Viral (Coxsackie, echovirus, adenovirus, CMV, mumps, varicella)
- B. Pertussis
- C. Brucellosis
- D. Rickettsial
- E. All of the above

**33. Which of the following statement regarding Cabot rings is correct?**

- A. May be seen in megaloblastic anemia within reticulocytes.
- B. It is a remnant of extra copy of RNA
- C. Frequently present in acute myeloid leukemia
- D. None of the above

**34. Splenic atrophy may be seen in the following, EXCEPT:**

- A. Celiac disease
- B. Aplastic anemia
- C. Crohn's disease
- D. Systemic lupus erythematosus (SLE)
- E. Ulcerative colitis

**35. A peripheral blood smear from a patient shows acanthocytes (spur cells) and echinocytes (burr cells). The most likely diagnosis is:**

- A. Combined liver and renal failure
- B. Lead poison
- C. G6PG deficiency
- D. Pyruvate kinase deficiency
- E. Combine TTP and G6PG deficiency

- 36. Which of the following diseases is associated with low reticulocyte counts including:**
- A. Aplastic anemia
  - B. Myelodysplasia
  - C. Anemia of chronic disease
  - D. Iron deficiency anemia
  - E. All of the above
- 37. How many mg of iron are in 1 ml of whole blood in a patient with a normal hematocrit?**
- A. 0.1 mg/ml
  - B. 0.3 mg/ml
  - C. 0.5 mg/ml
  - D. 1.0 mg/ml
  - E. 1.5 mg/ml
- 38. Which of the following statements is correct regarding iron studies in sideroblastic anemia?**
- A. Serum iron level is decreased
  - B. Total iron binding capacity is markedly increase
  - C. Percentage of transferrin saturation is increased
  - D. Decreased bone marrow iron storage
  - E. None of the above
- 39. Which of the following disorders is NOT associated with megaloblastic anemia?**
- A. Glutamate formiminotransferase deficiency
  - B. Dihydrofolate reductase deficiency
  - C. Lesch-Nyhan syndrome
  - D. Niemann-Pick syndrome
- 40. Which of following RBC morphologies may be observed in a peripheral blood smear of a patient with abetalipoproteinemia?**
- A. Acanthocytes
  - B. Stomatocytes
  - C. Pyknocytosis
  - D. Echinocytes
  - E. Elliptocytes
- 41. Pitting function of spleen is:**
- A. Dented RBCs make them easy to pass the splenic sinus
  - B. Removal of denatured hemoglobin such as Heinz body
  - C. Cause of basophilic stippling
  - D. Removal of Cabot ring
  - E. All of the above

- 42. What percent of platelets normally reside in the spleen?**
- A. 5%
  - B. 10%
  - C. 30%
  - D. 50%
  - E. 60%
- 43. Which of the following diseases is NOT commonly associated with florid follicular hyperplasia?**
- A. Rheumatoid arthritis
  - B. Syphilis
  - C. HIV-1 infection (early stage)
  - D. Castleman disease
  - E. Whipple disease
- 44. A chronic hemolytic anemia patient suddenly develops aplastic crisis with a low reticulocyte count. What is the most likely cause?**
- A. Progression to acute leukemia
  - B. Parvovirus B19 infection
  - C. Acute hemolytic anemia
  - D. Have not been transfused for 2 weeks
  - E. None of the above
- 45. Which of the following iron study results would you expect for a patient who has iron deficiency and active Crohn's disease?**
- A. Increased percentage transferrin saturation
  - B. Decreased soluble serum transferrin receptor
  - C. Normal or increased serum ferritin
  - D. Decrease total iron binding capacity
  - E. None of the above
- 46. Which of the following statements regarding serum transferrin level is correct?**
- A. Decreased in thalassemia
  - B. Increased in chronic infections
  - C. Increased in malignancy
  - D. Increased iron poisoning
  - E. Increased in hemolytic anemia
- 47. The normal range of serum iron in a male is:**
- A. 10-40 mcg/dL
  - B. 55-160 mcg/dL
  - C. 170-230 mcg/dL
  - D. 240- 300 mcg/dL
  - E. 330-550 mcg/dL

- 48. Which component contains most iron in the body?**
- A. Hemoglobin
  - B. Myoglobin
  - C. Ferritin
  - D. Hemosiderin
  - E. Transferrin
- 49. Which of the following conditions is NOT associated with an increased serum iron?**
- A. Following a high-iron meal
  - B. Hemochromatosis and liver disease
  - C. Infectious tuberculosis
  - D. Thalassemia
  - E. Pernicious anemia
- 50. Which of the following statements regarding idiopathic pulmonary hemosiderosis is correct?**
- A. Adult patients are common than children
  - B. May cause iron deficiency anemia
  - C. Repeat alveolar hemorrhage is rare
  - D. Presence of hemosiderin-loaded macrophages is diagnostic for this disease
  - E. All of the above
- 51. Which of the following statements regarding hereditary spherocytosis is correct?**
- A. Reticulocytosis is absent
  - B. Autosomal recessive inheritance pattern
  - C. Coombs test positive
  - D. Splenectomy is treatment of choice for severe disease
  - E. Decreased mean corpuscular hemoglobin concentration
- 52. The lifelong synthesis of anti-EBV IgG antibody and low level production of T-cells prevents against the recurrence of EBV infection by keeping virus infected B-cells from circulating. Which T-cell subset play an important role in the prevention recurrent EBV infection?**
- A. Early cytoplasmic CD3+ T-cells
  - B. CD4-, CD8- T-cells
  - C. CD4+, CD8- T-cells
  - D. CD4-, CD8+ T-cells
  - E. CD3+, CD10+ T-cells



- 53. What is the immunophenotype of the atypical lymphocytes found in infectious mononucleosis?**
- A. CD8+, CD4- T-cells
  - B. CD4+, CD8- T-cells
  - C. CD4-, CD8- T-cells
  - D. CD4+, CD8+ T-cell
  - E. None of the above
- 54. The antibody pattern of EBV infection is:**
- A. Positive IgM anti-VGA, high titer IgG anti-VGA, and anti-EA indicate an acute EBV infection
  - B. Negative IgM anti-VGA, high titer IgG anti-VGA, and anti-EA indicate an acute EBV infection
  - C. Positive IgM anti-VGA, high titer IgG anti-VGA, and anti-EA indicate a remote EBV infection
  - D. Negative IgG VGA, IgG EBNA, and IgM VGA antibody indicate a remote EBV infection
  - E. Positive IgG VGA, IgG EBNA and negative IgM VGA antibody indicate an acute EBV infection
- 55. Which antigen is associated with infectious mononucleosis?**
- A. Anti-i
  - B. Anti-P
  - C. Anti-I
  - D. Anti-e
  - E. Anti-Pl-A1
- 56. In EBV-induced infectious mononucleosis, the circulating atypical lymphocytes are predominantly:**
- A. CD8 positive T-cells
  - B. CD4 positive T-cells
  - C. CD20 positive B-cells
  - D. TdT positive precursor B-cells
  - E. Plasma cells
- 57. Hemophagocytic syndrome may be associated with which one of the following agents?**
- A. EBV
  - B. Babesia microti
  - C. Cryptococcus neoformans
  - D. Parvovirus 19
  - E. All of the above

- 58. Which of the following conditions is NOT associated with thrombocytopenia and large platelets?**
- A. Bernard-Soulier syndrome
  - B. May-Hegglin anomaly
  - C. Gray platelet syndrome
  - D. Wiskott-Aldrich syndrome
  - E. Montreal platelet syndrome
- 59. Which of the following has an abnormal response to ristocetin-induced platelet aggregation?**
- A. Bernard-Soulier
  - B. Glanzmann's thrombasthenia
  - C. Chediak-Higashi syndrome
  - D. Aspirin
  - E. None of the above
- 60. Which one of the following disorders DOES NOT show an increased ZPP (FEP)?**
- A. Iron deficiency
  - B. Anemia of chronic disease
  - C. Lead poison
  - D. Some sideroblastic anemia
  - E. Thalassemia
- 61. Donath-Landsteiner antibody is:**
- A. An anti-P antibody seen in paroxysmal cold hemoglobinuria
  - B. IgG biphasic hemolysin that binds at low temperature and lysis at room temperature
  - C. An anti-light chain antibody
  - D. A and B
  - E. None of the above
- 62. The best indication of intravascular hemolysis is:**
- A. Low haptoglobin
  - B. Low hemoglobin
  - C. Low hematocrit
  - D. Low bilirubin
  - E. Low plasma hemoglobin
- 63. Which of the following conditions may associate with pancytopenia and hypercellular marrow in a young person?**
- A. Paroxysmal nocturnal hemoglobinuria (PNH)
  - B. Fanconi anemia
  - C. CMV infection
  - D. Dyskeratosis congenita
  - E. Chronic arsenic poison

- 64. Thrombocytopenia with giant platelets is Not associated with:**
- A. May-Hegglin anomaly
  - B. Bernard-Soulier syndrome
  - C. Idiopathic thrombocytopenic purpura (ITP)
  - D. Mediterranean macrothrombocytosis
  - E. Thrombotic thrombocytopenic purpura (TTP)
- 65. An adult patient presents with GI bleeding, laboratory test showed increased aPTT and PT. You are concerned about the possibility of a factor X deficiency, what would be the next appropriate step?**
- A. Russell's venom time test
  - B. Perform an abdominal fat pad biopsy
  - C. Check factor VIII level
  - D. Check von Willebrand molecular polymer profile
  - E. Reassure patient, send patient home
- 66. Which stage of Hodgkin disease in a patient would be classified, who has above the diaphragm, in the spleen, and with B symptoms?**
- A. I
  - B. II
  - C. III
  - D. IV
  - E. V
- 67. A girl presents with hemarthrosis, and a history of bleeding disorder. Which factor deficiency is most likely?**
- A. von Willebrand factor (vWF)
  - B. Prekallikrein
  - C. Factor VIII
  - D. Factor XII
  - E. High molecular weight kininogen
- 68. DDAVP is useful in treating following bleeding disorder:**
- A. vWD type I
  - B. vWD type IIA
  - C. vWD type IIB
  - D. vWD type IIM
  - E. vVD type III
- 69. A 33-year-old woman had a sudden bleeding episode a few days ago. She has no history of bleeding tendencies. Her PTT is increased, physical examination is unremarkable, the patient is not stressed or on anticoagulant therapy. Laboratory studies show no correction of PTT with a mixed plasma study. The factor VIII activity is very low. The most likely cause of this patient's bleeding problem is:**
- A. Lupus anticoagulant present
  - B. Prekallikrein deficiency
  - C. Factor VIII inhibitor present
  - D. Factor XII deficiency
  - E. High molecular weight kininogen deficiency

- 70. Which of the following is a feature of Factor VIII:C:**
- A. Not part of Factor VIII macromolecular complex
  - B. Responsible for coagulant activity in conversion of X to Xa
  - C. Measured by PT assay for its activity
  - D. Does not use vWF as a carrier
  - E. not related to VIII:Ag level
- 71. Which of the following correctly match the electrophoresis findings with the type of delta/beta thalassemia?**
- A. Hb Lepore: 0% HbA, 0% HbA2, 5-15% Hb Lepore and 75% HbF
  - B. Heterozygous: 50% HbA, 10% HbA2 and 40% HbF
  - C. Homozygous: 0% HbA, 0% HbA2 and 100% HbF
  - D. Heterozygous: 80-90% HbA, 3% HbA2 and 5-20% HbF
  - E. A, C and D
- 72. Which of the following drugs is associated with immune-mediated hemolysis?**
- A. Melphalan
  - B. Quinidine
  - C. Sulfonamides
  - D. Chlorpromazine
  - E. All of the above
- 73. Which statement is correct, regarding Factor IX:**
- A. Synthesized in hepatocytes
  - B. Gene located on X chromosome
  - C. Vitamin K dependent serine protease
  - D. Binds effectively to collagen IV *in vitro*
  - E. All of the above
- 74. Which statement is correct regarding fibrinogen?**
- A. Afibrinogenemia is associated with a bleeding tendency
  - B. Plasma fibrinogen is synthesized in the liver
  - C. Fibrinogen is an acute-phase reactant
  - D. Thrombin cleave fibrinogen to fibrin
  - E. All above
- 75. Where is vWF made?**
- A. Endothelial cells
  - B. Megakaryocytes
  - C. Hepatocytes
  - D. A and B
  - E. B and C



- 76. Which coagulation factors are most likely to be elevated in liver disease?**
- A. AT III
  - B. Fibrinogen
  - C. Factor V
  - D. Factor VIII
  - E. All of the above
- 77. Which one of the following statements regarding Heparin Cofactor II is correct?**
- A. It is a serine protease inhibitor
  - B. Its inhibitory activity is enhanced by dermatan sulfate
  - C. Inhibits thrombin *in vivo* and *in vitro*
  - D. Deficiency results in venous thrombosis in most cases
  - E. All of the above
- 78. Which factor deficiency is least likely to be associated with thrombosis?**
- A. Protein C
  - B. Protein S
  - C. Factor XII
  - D. Fibrinogen (dysfibrinogenemia)
  - E. Factor XIII
- 79. A platelet aggregation demonstrates that Ristocetin-induced platelet aggregation is normal, and that collagen, arachidonic acid, and ADP studies were non-reactive. Which of the following is the most likely diagnosis?**
- A. Bernard-Soulier disease
  - B. Glanzmann thrombasthenia
  - C. Von Willebrand's disease
  - D. Aspirin use
  - E. Normal study
- 80. Which of the following is correct platelet aggregation pattern for afibrinogenemia (autosomal recessive)?**
- A. Adrenalin-induced aggregation is absent
  - B. ADP-induced aggregation is near normal or slightly decreased
  - C. Collagen-induced aggregation is near normal or slightly decreased
  - D. Ristocetin-induced aggregation is normal
  - E. All of the above

- 81. Which of the following statements regarding methyldopa-induced hemolysis is correct?**
- A. More than 95% of patients develop an IgM autoantibody
  - B. It is dose dependent
  - C. Methyldopa forms an immune complex, attaching to the RBC surface
  - D. Direct antiglobulin test (DAT) is usually negative
  - E. All of the above
- 82. An infant has severe normocytic and normochromic anemia, and lack of erythroid precursors in bone marrow. The most likely diagnosis is:**
- A. Dawn syndrome
  - B. Infection
  - C. Diamond-Blackfan syndrome
  - D. Fanconi aplastic anemia
  - E. Schwachman-Diamond syndrome
- 83. Which of the following is NOT a feature of hereditary spherocytosis?**
- A. Spherocytes on peripheral blood smear
  - B. Increased lysis with osmotic fragility tests
  - C. The common red cell cytoskeleton defects in autosomal dominant patient are ankyrin and b-spectrin
  - D. Autosomal recessive pattern is predominant pattern
  - E. The common red cell cytoskeleton defects in autosomal recessive patient are a-spectrin and protein 4.2
- 84. Which of the following statements regarding sideroblastic anemia is correct?**
- A. It has a dimorphic red cell population
  - B. It has reduced hemoglobin synthesis because of failure to incorporate heme into protoporphyrin to form hemoglobin iron accumulates, particularly in mitochondria
  - C. Sideroblastic anemia can be present in chronic alcoholism and lead poisoning
  - D. Mean corpuscular volume is usually normal or slightly increased, but occasionally low, leading to confusion with iron deficiency
  - E. All of the above
- 85. Which condition is associated with stomatocytosis?**
- A. Rh null disease
  - B. Hereditary stomatocytosis
  - C. Alcoholics with liver disease or obstructive liver disease
  - D. Artifact of drying/low pH
  - E. All of the above

- 86. Acquired stomatocytosis can be present in the following condition:**
- A. Patient receiving vinblastine therapy
  - B. Patient receiving chlorpromazine therapy
  - C. Patient who is an active alcoholic
  - D. Patient who has hepatobiliary disease
  - E. All of the above
- 87. Sick cells and target cells are seen in a patient's peripheral blood smear, the most likely diagnosis is:**
- A. Sick cell disease
  - B. Thalassemia
  - C. Hemoglobin C
  - D. A and B
  - E. A and B, or A and C
- 88. A patient's hemoglobin electrophoresis showed: 29% HbS, 2% HbA<sub>2</sub>, and 69% HbA, what is the most likely diagnosis?**
- A. Alpha thalassemia-Sickle cell trait
  - B. Sick cell trait
  - C. Sick cell disease and thalassemia Beta<sup>0</sup>
  - D. Sick cell disease and thalassemia Beta<sup>+</sup>
  - E. Sick cell disease
- 89. What is the consequence of an abnormal hemoglobin that does not bind 2,3 DPG?**
- A. Decreased O<sub>2</sub> affinity
  - B. Decreased RBC count
  - C. Shift of O<sub>2</sub> dissociation curve to the left
  - D. Decreased delivery of O<sub>2</sub> to the tissues
  - E. All of the above
- 90. Which enzyme in the heme synthesis pathway is inhibited by lead?**
- A. Aminolevulinic acid dehydratase (ALA dehydratase)
  - B. Uroporphyrinogen I synthase
  - C. Uroporphyrinogen decarboxylase
  - D. Coproporphyrinogen III oxidase
  - E. Protoporphyrinogen IX oxidase
- 91. Which of the following disorders DOES NOT have an increased FEP?**
- A. Iron deficiency anemia
  - B. Sideroblastic anemia
  - C. Lead poison
  - D. Anemia of chronic disease
  - E. Thalassemia

- 92. Which of the following statements regarding pyruvate kinase deficiency is correct?**
- A. Decrease spiculated spheroid cells on the peripheral blood smear after splenectomy
  - B. Low levels of pyruvate kinase activity in white cells
  - C. Positive Coombs test
  - D. Bone marrow examination reveals erythroid hyperplasia and active marrow
  - E. All of the above
- 93. The father has a genotype of -A/AA and mother has a genotype of -A/-A, what percentage of children will have the alpha-thalassemia trait?**
- |        |        |
|--------|--------|
| A. 5%  | B. 15% |
| C. 25% | D. 40% |
| E. 50% |        |
- 94. A patient's hemoglobin electrophoresis showed: HbS 59%, HbA 32%, F 3%, A2 6%. The most likely diagnosis is:**
- A. Alpha thalassemia-Sickle cell trait
  - B. Sickle cell trait
  - C. Sickle cell disease and thalassemia Beta<sup>0</sup>
  - D. Sickle cell disease and thalassemia Beta<sup>+</sup>
  - E. Sickle cell disease
- 95. The multimer analysis pattern of type I von Willebrand disease multimer analysis is:**
- A. Normal pattern, but decreased in quantity
  - B. Loss of intermediate and high molecular weight multimer
  - C. Loss of high molecular weight multimer
  - D. Total absent
  - E. Normal pattern, no decreased in quantity
- 96. What are features used to distinguish S-Beta<sup>+</sup> thalassemia from S-Beta<sup>0</sup> thalassemia?**
- A. S-Beta<sup>+</sup> thalassemia has positive sickling test, S-Beta<sup>0</sup> thalassemia is negative
  - B. S-Beta<sup>+</sup> thalassemia has some hemoglobin A, S-Beta<sup>0</sup> thalassemia has zero
  - C. S-Beta<sup>+</sup> thalassemia has some hemoglobin S, S-Beta<sup>0</sup> thalassemia has zero



- D. S-Beta<sup>+</sup> thalassemia has megaloblastic changes, S-Beta<sup>0</sup> thalassemia does not
  - E. S-Beta<sup>+</sup> thalassemia has some hemoglobin C, S-Beta<sup>0</sup> thalassemia has zero
- 97. An infant with high levels of hemoglobin F, and adult with hereditary persistence of fetal hemoglobin (HPFH) both undergo testing. Which of the following would be the expected test result?**
- A. Kleihauer-Betke test shows two populations of RBCs (heterogenous distribution) in the infant, and a homogeneous distribution in the adult with HPFH
  - B. Kleihauer-Betke test shows two populations of RBCs (heterogenous distribution) in in the adult with HPFH and a homogeneous distribution in the infant
  - C. Kleihauer-Betke test shows two populations of RBCs (heterogenous distribution) in both the infant and the adult with HPFH
  - D. Kleihauer-Betke test shows a homogeneous distribution of RBCs in the infant and in the adult with HPFH
- 98. The Prussian blue positive inclusions in ringed sideroblasts are located in the:**
- A. Golgi apparatus
  - B. Mitochondria
  - C. Rough endoplasmic reticulum
  - D. Smooth endoplasmic reticulum
  - E. Melanosome
- 99. Which of the following disorders DOES NOT have an increased ESR?**
- A. Increased fibrinogen
  - B. Sickle cell disease
  - C. Monoclonal gammopathy
  - D. Autoimmune related diseases
  - E. Neoplastic diseases
- 100. What is the clinical presentation of dysfibrinogenemia?**
- A. Mostly asymptomatic
  - B. Hemarthron
  - C. Severe bleeding
  - D. Thrombosis
  - E. Anemia

**101. Which of the following factor deficiencies is NOT associated with an autosomal recessive inheritance pattern?**

- A. Wiskott-Aldrich
- B. Alpha2-plasmin
- C. Glanzmann's thrombasthenia
- D. Bernard-Soulier's disease
- E. Chediak-Higashi

**102. Which of the following factor deficiencies will result in bleeding?**

- A. XII (Hageman)
- B. Prekallikrein
- C. HMW kininogen
- D. XI

**103. Which of the following hemoglobins does not have a high oxygen affinity hemoglobin?**

- A. Hb Chesapeake
- B. Hb Seattle
- C. Hb Malmo
- D. Hb Hiroshima
- E. Hb Bethesda

**104. Which parental genotypes can give rise to a child with HbH disease?**

- A. Alpha, alpha/-,- and alpha, -/alpha, alpha
- B. Alpha, alpha/-,- and alpha, alpha constant spring/-,-
- C. Alpha, alpha/alpha,- and alpha, alpha/alpha,-
- D. A and B
- E. A, B and C

**105. Which factors are at normal levels at birth?**

- A. II
- B. III
- C. V
- D. IX
- E. X

**106. A patient presents with a lifelong history of bleeding tendency. Laboratory tests showed a normal aPTT and a prolonged PT. The prolonged PT was corrected by normal serum but not by barium sulfate-absorbed plasma. What is the most likely factor deficiency?**

- A. Factor V
- B. Factor VII
- C. Factor VIII
- D. Factor XI
- E. Factor XII

**107. A patient presented as a lifelong history of a mild or severe bleeding tendency, laboratory tests showed a normal Russell's viper venom time and a prolonged PT. What is the most likely factor deficiency?**

- A. Factor V
- B. Factor VII
- C. Factor VIII
- D. Factor XI
- E. Factor XII

**108. Which of the following can give a positive sickling test?**

- A. Hemoglobin C<sub>Georgetown</sub>
- B. Hemoglobin C<sub>Harlem</sub>
- C. Sick cell trait
- D. Hemoglobin I
- E. All of the above

**109. Point mutation associated with sickle hemoglobin results in which of the following changes on the  $\beta$ -globin chain?**

- A. 6 glutamic acid to valine
- B. 6 glutamic acid to lysine
- C. 26 glutamic acid to valine
- D. 26 glutamic acid to lysine
- E. None of the above

**110. Which of the following statements is correct regarding familial LCAT (Lecithin-cholesterol acyltransferase) deficiency?**

- A. Causes brain injury
- B. Autosomal dominant inheritance
- C. Severe neutropenia
- D. The major morbidity and mortality is related to renal failure
- E. All of the above

**111. In which condition the reptilase time (RT) and thrombin time (TT) will be prolonged:**

- A. DIC
- B. Dysfibrinogenemia
- C. Heparin
- D. A and B
- E. A, B and C

**112. Which of the following statements regarding Wiskott-Aldrich syndrome is correct:**

- A. Is characterized by eczema, thrombocytopenia, and recurrent infections
- B. Wiskott-Aldrich syndrome protein (WASP) is located on Xp11.22 and is inherited as X-linked recessive
- C. IgM levels usually are low, whereas IgG and IgA levels can be normal or elevated
- D. with increasing age, patients become lymphopenic and have severely impaired cell-mediated immunity
- E. All of the above

**113. Which of the following disorders is NOT due to defective DNA repair?**

- A. Fanconi anemia
- B. Bloom's syndrome
- C. Ataxia telangiectasia
- D. Xeroderma pigmentosa
- E. Wiskott-Aldrich syndrome

**114. Clinical features and peripheral blood smear findings associated with vitamin E deficiency include:**

- A. Hemolytic anemia
- B. Thrombocytosis
- C. Edema of the dorsum of the feet and pretibial area
- D. Increase in reticulocytes, small dense poikilocytes, and keratocytes (bitten cells) seen on peripheral blood smear
- E. All of the above

**115. Which of the following factor deficiencies DOES NOT cause thrombosis?**

- |                     |              |
|---------------------|--------------|
| A. Antithrombin III | B. Protein C |
| C. XII (Hageman)    | D. IX        |
| E. Factor V Leiden  |              |

**116. Which vitamin K-dependant factor has the shortest half-life?**

- A. Prothrombin (factor II)
- B. Factor VII
- C. Factor IX (deficiency in hemophilia B)
- D. Factor X
- E. Protein C



**117. Which coagulation factors can become deficient in nephrotic syndrome?**

- |        |         |
|--------|---------|
| A. II  | B. V    |
| C. VII | D. VIII |
| E. IX  |         |

**118. Which of the following disorders DOES NOT have Heinz bodies in RBC?**

- A. Glucose-6-phosphate dehydrogenase deficiency
- B. Oxidizing drugs
- C. Unstable hemoglobins
- D. Chemical poison
- E. Spherocytosis

**119. Which of the following coagulation factors is NOT increased during pregnancy?**

- |               |        |
|---------------|--------|
| A. VIII       | B. vWF |
| C. II         | D. VII |
| E. Fibrinogen |        |

**120. Which of the following is associated with May-Hegglin anomaly?**

- A. Autosomal recessive inheritance pattern
- B. Abnormal platelet studies
- C. Döhle bodies only present in the monocytes
- D. Thrombocytopenia and giant platelets in peripheral smear
- E. Heinz bodies present in all granulocytes

**121. Which of the following disorders DOES NOT have an increased risk of develop leukemias, deficiency in DNA repair mechanism and instability?**

- A. Fanconi anemia
- B. Bloom's syndrome
- C. Xeroderma pigmentosum
- D. Chediak-Higashi syndrome
- E. Ataxia telangiectasia

**122. Gray platelet syndrome, is lack characterized by an isolated deficiency of:**

- A. Alpha granules
- B. Beta granules
- C. Delta granules
- D. Lysosomes
- E. Zeta granules

**123. Which statement regarding transcobalamin I, II, III is correct?**

- A. Transcobalamin II is synthesized in granulocytes
- B. Transcobalamin I binds most of the newly absorbed B<sub>12</sub>
- C. Transcobalamin III does not significantly bind B<sub>12</sub>
- D. Transcobalamin I is synthesized in the liver
- E. Transcobalamin II deficiency does not result in megaloblastic anemia

**124. Which enzyme deficiency in the anaerobic RBC pathway is NOT associated with hemolysis?**

- A. Hexokinase
- B. Glucosephosphate isomerase
- C. Phosphofructokinase
- D. Triosephosphate isomerase
- E. Enolases

**125. Which factor deficiency may be associated with Hodgkin lymphoma?**

- A. II
- B. V
- C. VII
- D. VIII
- E. IX

**126. Which of the following statements about factor V is INCORRECT?**

- A. A cofactor from liver or megakaryocytes
- B. Activated by thrombin, along with Xa, Ca<sup>++</sup>
- C. Inactivated by activated protein C (APC)
- D. Some factor V is stored in platelet dense granules
- E. An Arg to Gln at codon 506 mutation in factor V leads to resistance to inactivation by APC (factor V Leiden)

**127. Which of the following is NOT a feature of thrombocytopenia with absent radii (TAR) syndrome?**

- A. Low to moderate platelet count, usually in 15-50K range
- B. Decreased number of megakaryocytes in the bone marrow
- C. Skin bruising
- D. Despite platelet transfusion and other supportive treatment, most patients will die after 1 year of life
- E. Peripheral left-shifted leukocytosis

- 128. A patient on cardiac bypass was given heparin followed by protamine. After protamine, the TT was normal but the PT was still prolonged, what is the possible explanation?**
- A. Heparin rebound
  - B. Laboratory mistake
  - C. Protamine neutralized heparin but caused platelet dysfunction
  - D. Excessive heparin and platelet dysfunction due to blood circulating through oxygenator pump
  - E. None of the above
- 129. Which of the following drugs may cause hemolysis in a patient with G6PD deficiency?**
- A. Acetanilide, Methylene blue
  - B. Pentaquine
  - C. Sulfacetamide
  - D. Trinitrotoluene (TNT)
  - E. All of the above
- 130. Which of the following is NOT feature of Vitamin E deficiency in a premature infant?**
- A. Bleeding
  - B. Anemia
  - C. Skin rash
  - D. Edema
  - E. Thrombocytosis
- 131. Which of the following statements regarding hereditary pyropoikilocytosis (HPP) is correct?**
- A. There is a strong relation between hereditary elliptocytosis and HPP
  - B. Peripheral blood morphology is similar to hereditary spherocytosis
  - C. No erythrocyte membrane protein defect has been identified so far
  - D. HPP is most commonly seen in European descendants
  - E. None of the above
- 132. Which of the following factors is associated with a decreased 2,3-DPG level?**
- A. 2,3 DPG mutase deficiency
  - B. 2,3 DPG phosphatase deficiency
  - C. Glucose phosphate isomerase deficiency
  - D. A and B
  - E. A, B and C

**133. The cause of march hemoglobinuria is:**

- A. G6PD enzyme defect
- B. Repetitive trauma causing hemolysis
- C. Heme synthesis deficiency
- D. Postinfectious glomerulonephritis
- E. Systemic lupus erythematosus

**134. The enzyme defect in Gaucher's disease is:**

- A. Galactosylceramidase
- B. Alpha-Galactosidase A
- C. Glucocerebrosidase
- D. Sphingomyelinase
- E. Alpha 1,4-Glucosidase

**135. Which of the following statements is NOT associated with Fanconi anemia?**

- A. Autosomal dominant inherited pattern
- B. Skeletal abnormalities
- C. Increased risk of leukemia
- D. Mental retardation
- E. Skin pigmentation

**136. Which of the following regarding cold agglutinin disease is correct?**

- A. Idiopathic cold agglutinin disease may be associated with low-grade lymphoma
- B. Mycoplasma infection (anti-I), infectious mononucleosis (Anti-i) are IgM autoantibodies that react and bind below 37 degrees
- C. Donath-Landsteiner antibody is associated with Paroxysmal Cold Hemoglobinuria (PCH)
- D. Waldenström macroglobulinemia may be associated with cold agglutinin disease
- E. All of the above

**137. Which of the following regarding hemoglobin Lepore disorder is correct?**

- A. Hemoglobin Lepore is a composite delta beta chain that is produced slowly resulting in a hypochromic microcytic erythrocytes
- B. Approximately 20% of the hemoglobin is of the Lepore type and 80% is fetal hemoglobin, Hemoglobins A and A2 are absent
- C. Hemoglobin Lepore migrates like Hb S on alkaline electrophoresis and with Hb A on acid electrophoresis



- D. In the heterozygous state, the findings are similar to those of thalassemia minor and in homozygous state, the findings are similar to those of thalassemia major
- E. All of the above

**138. Hemoglobin G-Philadelphia is a:**

- A. Alpha chain defect
- B. Delta chain defect
- C. Beta chain defect
- D. Gamma chain defect
- E. None of the above

**139. Which of the following statements regarding Hemoglobin G Philadelphia is correct?**

- A. Result of asparagine to lysine substitution at 68th position of the alpha globin chain
- B. Common in African-American population
- C. Present of Hb-G2 band on the alkaline electrophoresis or isoelectric focusing is helpful for the diagnosis
- D. Hemoglobin G-Philadelphia has no clinical or hematological effects
- E. All of the above

**140. Which of the following hemoglobinopathies will migrate in the Hb S zone on alkaline electrophoresis?**

- |           |      |
|-----------|------|
| A. H      | B. D |
| C. Bart's | D. C |
| E. O      |      |

**141. Which factors are removed by barium sulfate or aluminum hydroxide from plasma?**

- A. II
- B. VII
- C. IX
- D. None of the above
- E. All of the above

**142. Which factors are present in aged normal serum?**

- A. VII
- B. IX
- C. X
- D. None of the above
- E. All of the above

**143. Which of the following statement regarding juvenile myelomonocytic leukemia (JMML) is correct?**

- A. Peripheral blood monocyte count less than  $1 \times 10^9/L$
- B. BCR-ABL1 fusion gene present
- C. GMS-CSF hyposensitivity of myeloid progenitors *in vitro*
- D. May have mutations involving genes of RAS/MARK pathway
- E. More than half of JMML patients have clinical diagnosis of neurofibromatosis 1 (NF1)

**144. Which of the following is NOT a feature of juvenile myelomonocytic leukemia (JMML)?**

- A. -5, 5q are common
- B. No Ph chromosome present
- C. Marked male predominance
- D. Skin rash, extramedullary infiltrates and recurrent infections.
- E. Increased Hb F

**145. Juvenile myelomonocytic leukemia (JMML) is associated with:**

- A. Monosomy 5q syndrome
- B. Female predominance
- C. Neurofibromatosis type 1 (10-15%)
- D. Decreased HbF
- E. Low incidence of evolution to acute leukemia

**146. Leukoerythroblastic reaction most commonly seen in:**

- A. Hairy cell leukemia
- B. Metastatic carcinoma to bone marrow
- C. Large granular lymphocytic leukemia
- D. Chronic lymphocytic leukemia
- E. Parvovirus infection

**147. Which of the following is correct regarding leukocyte alkaline phosphatase (LAP) score:**

- A. Increased in CML
- B. Decreased in infection
- C. May be normal in remission CML
- D. Markedly decreased in remission CML
- E. All of the above

**148. Which of the following conditions have a low LAP?**

- A. Leukemoid reaction due to infection
- B. Polycythemia vera

- C. Chronic myeloid leukemia
- D. Hodgkin lymphoma
- E. Acute lymphoblastic leukemia

**149. Vacuolization of marrow erythroblasts can be seen in:**

- A. Alcohol
- B. Chloramphenicol
- C. Neoplastic erythroblasts
- D. All of the above

**150. An MDS patient with a bone marrow blast count of 7%, 14% ringed sideroblasts, and no Auer rods identified, should be classified as:**

- A. Normal
- B. Refractory anemia (RA)
- C. Refractory anemia with excess blasts (RAEB) type 1
- D. Refractory anemia with excess blasts (RAEB) type 2
- E. Acute leukemia

**151. The EPO level in polycythemia vera is:**

- A. Markedly increased
- B. Markedly decreased
- C. Normal
- D. Slightly increased
- E. Slightly decreased

**152. Which of the following conditions will cause absolute polycythemia secondary to EPO production?**

- A. Hepatocellular carcinoma
- B. Renal cell carcinoma
- C. Cerebellar hemangioma
- D. Lung tumors
- E. All of the above

**153. Which subtype of AML has the high risk of CNS and extramedullary involvement (skin rash)?**

- A. AML with minimal differentiation
- B. AML without maturation
- C. AML with maturation
- D. Acute myelomonocytic leukemia
- E. Acute erythroid leukemia

**154. Topoisomerase II inhibitor therapy-related acute leukemia is frequently associated with:**

- A. t(9;22)
- B. 11q23
- C. -5q
- D. trisomy 12
- E. t(8;22)

**155. Acute leukemia with t(4;11)(q21;q23) translocation usually associated with:**

- A. Acute mixed lineage leukemia
- B. Acute erythroid leukemia
- C. Acute megakaryoblastic leukemia
- D. Acute basophilic leukemia
- E. Acute panmyelosis with myelofibrosis

**156. Which chromosomal abnormality is associated with therapy-related leukemia?**

- A. Monosomy 5 or 7
- B. 5q-
- C. 7q-
- D. 11q23
- E. All of the above

**157. Which of the following statements regarding chronic myelogenous leukemia BCR-ABL1 gene is correct?**

- A. Most cases have an 8.5 Kb mRNA BCR-ABL1 gene that produces a 210 Kb fusion protein which has tyrosine kinase activity
- B. BCR-ABL1 gene produces a 230 Kb fusion protein in a small number of cases
- C. BCR-ABL1 gene produces a 190 Kb fusion protein is frequently associated with ALL
- D. CML with a 190 Kb fusion protein has an increased number of monocytes, and it may be confused with CMML
- E. All of the above

**158. Which of the following statements regarding polycythemia vera is correct?**

- A. Iron is usually negative in the bone marrow
- B. EPO is markedly increased
- C. Hepatosplenomegaly is uncommon
- D. Artery O<sub>2</sub> saturation is increased
- E. All of the above



**159. Which of the following is NOT a feature of blast crisis CML?**

- A. Blasts are equal or great than 20% of the peripheral blood leukocytes or of the nucleated cells of bone marrow
- B. Approximately 20-30% cases are lymphoblasts
- C. In blast phase, alkaline phosphatase in neutrophils is markedly reduced
- D. p230 abnormal fusion protein is the most common
- E. Mixed phenotype acute leukemia is rare

**160. Which of the following statements regarding PAS stain is NOT correct?**

- A. Block positivity in immature erythroid of acute erythroid leukemia (M6)
- B. Diffuse positivity in mature erythroid cell
- C. Coarse positivity in ring sideroblasts
- D. Coarse positivity in acute megakaryoblastic leukemia
- E. Block positivity in lymphoblastic lymphomas/leukemias

**161. Which of the following statements regarding cytochemical stains for acute erythroid leukemia (M6) is correct?**

- A. Myeloblasts are MPO and SBB negative
- B. Erythroid cells are MPO and SBB positive
- C. Erythroid cells are PAS positive
- D. A and B
- E. None of the above

**162. Which of the following statements regarding acute megakaryoblastic leukemia (M7) is correct?**

- A. MPO and SBB positive
- B. PAS and acid phosphatase are usually positive
- C. NSE (naphthyl acetate) is usually negative
- D. CD41 is usually negative
- E. CD61 is usually negative

**163. Which of the following immunophenotype of early precursor B-cell ALL is INCORRECT?**

- A. CD19 positive
- B. TdT positive
- C. CD2 negative
- D. CD10 negative
- E. Cytoplasmic mu positive

**164. Which of the following translocations related to precursor B-ALL, is commonly seen in infant <1 year old?**

- A. t(9;22)(q34;q11)
- B. t(v;11)(v;q11)
- C. t(1;19)(q23;p13)
- D. t(5;14)(q31;q32)
- E. t(12;21)(p13;q22)

**165. The typical immunophenotype of Hairy cell leukemia is:**

- A. Surface Ig+, CD11c-, CD19+, CD25- and CD103-
- B. Surface Ig+, CD11c+, CD19+, CD25+ and CD103-
- C. Surface Ig+, CD11c+, CD19+, CD25+ and CD103+
- D. Surface Ig+, CD11c-, CD19+, CD25- and CD103+
- E. Surface Ig-, CD11c-, CD19+, CD25- and CD103+

**166. The typical immunophenotype of mantle cell lymphoma is:**

- A. Bright surface Ig, CD5+, CD20+, CD23-, FMC7+
- B. Dim surface Ig, CD5+, CD20+, CD23-, FMC7-
- C. Dim surface Ig, CD5+, CD20+, CD23+, FMC7-
- D. Bright surface Ig, CD5-, CD10+ CD20+, FMC7+
- E. Surface Ig negative, CD5-, CD20+, CD23-, FMC7+

**167. Which of the following immunophenotype of adult T-cell lymphoma/ leukemia is INCORRECT?**

- A. Rare cases are CD4-/CD8+
- B. Most cases are CD4+/CD8-
- C. CD7+
- D. CD2+
- E. Strong CD25 expression in majority of the case

**168. Which of the following features of enteropathy-type T-cell lymphoma (EATL) is correct:**

- A. Most of the cases have a monomorphic histological appearance
- B. Neoplastic T-cells are usually CD4 and CD5 positive
- C. +8q24 (MYC) is commonly seen in type II EATL
- D. Neoplastic T-cells are usually negative in type II EATL
- E. +9q31.3 or -16q12.1 are uncommon in both EATL and type II EATL

**169. Burkitt lymphoma translocation is:**

- A. t(11;14)
- B. t(14;18)
- C. t(2;8)
- D. t(9;22)
- E. t(8;21)

**170. Follicular lymphoma translocation is:**

- A. t(11;14)
- B. t(14;18)
- C. t(2;8)
- D. t(9;22)
- E. t(8;21)

**171. Which of the following is NOT a typical immunophenotypic features of Pre-B acute lymphoblastic lymphoma/leukemia?**

- A. HLA-DR+
- B. CD19+
- C. CD10 may be negative in infant
- D. Surface IgM+
- E. No light chain expression

**172. Which of the following cytogenetic findings is NOT associated with poor prognosis?**

- A. Hypodiploidy-near haploidy or near tetraploidy
- B. del 17
- C. t(9;22)
- D. t(12;21)
- E. t(4;11)

**173. Which of the following is NOT a feature of t(4;11)(q21;q23) leukemia?**

- A. The most common rearrangement in infant less than 1 year old
- B. Markedly elevated leukocyte counts, splenomegaly
- C. Poor prognosis
- D. Majority have an early B-cell precursor phenotype, usually CD10 positive
- E. Biphenotypic and bilinear leukemias are uncommon

**174. Which of the following statements is true, regarding the (9;22)(q34;q11) translocation:**

- A. Present in more than 90% of adult ALL
- B. Results in MLL-AF4 fusion gene
- C. p210 KD protein is commonly seen in ALL
- D. Result in a fusion gene with potent tyrosine kinase activity
- E. More common in pediatric ALL patients than adult ALL patients

**175. Which of the following statements regarding chronic lymphoproliferative disorder of NK cells is correct?**

- A. Surface CD3 positive and cytoplasmic CD3 negative
- B. Cytogenetic karyotype is normal in most of cases
- C. T-cell receptor gene rearrangement is positive in 40% of cases

- D. EBV is positive in most of cases
- E. Majority of patients have an aggressive clinical course

**176. Progressively transformed germinal center (PTGC) may be found in which of the following condition?**

- A. Mantel cell lymphoma
- B. Chronic lymphocytic lymphoma
- C. Burkitt's lymphoma
- D. Classic Hodgkin lymphoma, nodular sclerosing type
- E. Nodular lymphocyte predominant Hodgkin lymphoma

**177. The correct answer regarding post-transplantation lymphoproliferative disorder (PTLD) is:**

- A. PTLD is more frequent in renal transplantation than heart transplantation
- B. PTLD is common in extranodal sites
- C. EBV infection in PTLD is rare
- D. When immunosuppressive drugs are reduced and withdrawn, only rare cases of PTLD will experience remission
- E. Even if PTLD is monoclonal, multifocal, and has karyotypic abnormalities, less than 10% will die

**178. Which of the following descriptions of NK cell large granular cell leukemia is correct?**

- A. Most have an indolent course
- B. Frequent association with rheumatoid arthritis and autoimmune disease, polyclonal gammopathy or autoimmune antibody may present
- C. Gene rearrangement is germline
- D. CD2, CD3, CD7, CD4, CD8, CD56, and CD57 positive
- E. CD2, CD3, CD7, CD4, CD56, and CD57 positive

**179. The typical immunophenotype of neoplastic cell of the Sézary syndrome is:**

- A. CD2, CD3, CD4, CD5 positive; CD7 absent or weak; and CD8 negative
- B. CD2, CD3, CD4, CD5, CD25 positive; CD4, CD8 positive or CD4 and CD8 negative, CD7 absent or weak
- C. Bright surface Ig, CD19, CD20, CD22, CD11c positive; variable CD25 and CD103
- D. Bright surface Ig, CD19, CD20, CD22, CD10 positive
- E. CD2, CD5, CD4, CD7 positive; CD8 negative; involving 14q11 common



**180. The typical prolymphocytic variant of hairy cell leukemia is:**

- A. bright surface Ig, CD11c+, CD25+, CD103-, TRAP+
- B. bright surface Ig, CD11c+, CD25+, CD103+, TRAP+
- C. bright surface Ig, CD11c+, CD25-, CD103+, TRAP+
- D. bright surface Ig, CD11c-, CD25-, CD103-, TRAP-
- E. dim surface Ig, CD11c-, CD25-, CD103-, TRAP-

**181. Which of the following statements regarding angioimmunoblastic T-cell lymphoma (AITL) is correct?**

- A. Vascular proliferation with thickened or hyalinized wall is a specific diagnostic feature of AITL
- B. Both T and B gene rearrangement may be positive
- C. Expanded germinal center with reduced follicular dendritic cell meshwork
- D. Clusters of clear cells is not one of the features of AITL
- E. Patients usually have localized disease

**182. Which of the following statements is correct, regarding acute megakaryoblastic leukemia (AML-M7)?**

- A. Detection of platelet peroxidase in perinuclear cisternae and endoplasmic reticulum, by ultrastructural methods, may be the earliest method to identify AML-M7
- B. CD41, CD42, CD61 are specific for identification of AML-M7
- C. AML-M7 may associated with trisomy 21 and t(1;22) abnormalities
- D. AML-M7 can present as nonhematopoietic tumors with extra-medullary abdominal masses, osteolytic lesions, and extensive bone marrow fibrosis
- E. All the above

**183. Which of the following statements regarding Hodgkin lymphoma is correct?**

- A. Reed-Sternberg cells of classic Hodgkin lymphoma are usually CD15-, CD20+, CD30+, CD45+
- B. 2-5% of nodular lymphocyte predominant Hodgkin lymphoma progress to diffuse large B cell lymphoma
- C. Reed-Sternberg cells of classic Hodgkin lymphoma are usually Fascin negative
- D. Nodular lymphocyte predominant Hodgkin lymphoma has a bimodal age distribution
- E. All of the above

**184. The lineage of Reed-Sternberg cells is:**

- A. Granulocyte
- B. Monocyte
- C. T-cell
- D. B-cell
- E. Dendritic cell

**185. Which of the following statements regarding gamma heavy chain disease (HCD) is correct?**

- A. Lytic lesions are typically absent
- B. Both kappa and lambda light chains are present in plasma cells
- C. Mu HCD is considered as a variant of MALT lymphoma
- D. Alpha HCD is typically resembles of CLL
- E. None of the above

**186. Which of the following statements regarding infectious lymphocytosis (other than infectious mononucleosis and acute lymphoblastic leukemia) is correct?**

- A. Infectious lymphocytosis is usually composed of large atypical lymphocytes
- B. The lymphocytes of infectious lymphocytosis are mostly T-cells
- C. Infectious lymphocytosis usually are accompanied by anemia or thrombocytopenia or hepatosplenomegaly
- D. The lymphocytes of infectious lymphocytosis have an immature immunophenotype and are TdT positive
- E. Infectious lymphocytosis is usually caused by fungal infection

**187. Which of the following descriptions regarding CD4 and CD8 status is correct?**

- A. Most cases of mycosis fungoides are CD4+, CD8-
- B. Most cases of T-ALL are CD4+, CD8-
- C. Most cases of hepatosplenic T-cell lymphoma are CD4-, CD8-
- D. Most cases of T-prolymphocytic leukemia are CD4+, CD8-
- E. All of the above

**188. What are the most common surface Ig classes associated with CLL?**

- A. IgM>IgM+IgD>IgD
- B. IgD>IgM+IgD>IgM
- C. IgA>IgM+IgD>IgM
- D. IgG>IgE+IgD>IgM
- E. IgE>IgG+IgD>IgM

**189. A CLL patient has peripheral lymphocytosis and lymphadenopathy, no evidence of hepatosplenomegaly, hemoglobin >10 mg/dl, and normal platelet count. What is the patient's Rai stage?**

- A. 0
- B. I
- C. II
- D. III
- E. IV

**190. Which of the following statements regarding the prognosis of acute lymphoblastic leukemia/lymphoma (ALL) is correct?**

- A. B-ALL has a good prognosis in children, but is less favorable in adults
- B. < 1 year-old and >10-year-old have good prognosis
- C. High WBC count associated with good prognosis
- D. t(9;22) translocation in children has good prognosis
- E. None of the above

**191. Which of the following immunophenotypic findings are consistent with the diagnosis of CLL?**

- A. Dim or faint surface immunoglobulin, sIgM>sIgM+IgD>IgD
- B. Dim CD20, CD19 is strong than CD20
- C. Dim or faint CD11c can be positive.
- D. Trisomy 12 is common than 13q
- E. All of the above

**192. A small cell variant of Sézary cell is called:**

- A. Hashimoto cells
- B. Gaucher cells
- C. Lutzner cells
- D. Thomas cells
- E. None of the above

**193. The percentage of intracytoplasmic Mu heavy chains in pre-B lymphoblastic lymphoma is:**

- A. Less than 5%
- B. 5-10%
- C. 20-25%
- D. 50-60%
- E. Greater than 90%

**194. What is the most frequent type of primary CNS lymphomas?**

- A. Burkitt's lymphoma
- B. Diffuse large B-cell lymphoma
- C. Peripheral T-cell lymphoma
- D. Follicular lymphoma
- E. Mantle cell lymphoma

**195. Which of the following are TdT negative?**

- A. Hematogones
- B. Cortical thymocytes
- C. Lymphoblastic lymphoma/leukemia
- D. Some cases of blast crisis of CML
- E. Burkitt lymphoma

**196. Which leukemia/lymphoma is positive with CD5?**

- A. CLL/SLL
- B. Mantle cell lymphoma
- C. Some diffuse large cell lymphomas
- D. A and B
- E. A, B and C

**197. The most common site of extramedullary plasmacytoma is:**

- A. Head and neck
- B. Vertebral body
- C. Pelvis
- D. Humerus
- E. Femoral head

**198. Which one of the following is NOT a poor prognostic marker of multiple myeloma?**

- A. Severe anemia
- B. Poor performance rating
- C. Renal failure
- D. Mutated IgVH
- E. High beta2-microglobulin

**199. Favorable risk genetics by FISH in multiple myeloma is/are:**

- A. t(11;14) or t(6;14)
- B. Deletion 13
- C. t(4;14) or t(14;16) or t(14;20)
- D. Deletion 17p13
- E. Hypodiploidy

**200. Which type of myeloma has a low serum component, but has a high quantity of light chain in the urine?**

- A. IgA myeloma
- B. IgG myeloma
- C. IgM myeloma
- D. IgD myeloma
- E. IgE myeloma



**201. Which type of myeloma is most commonly associated with lambda light chain monoclonality?**

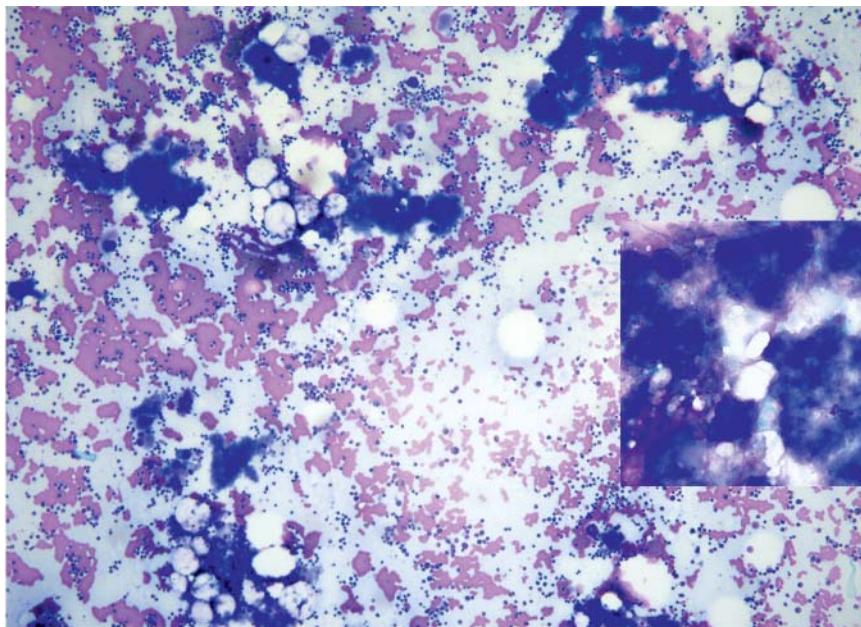
- A. IgA
- B. IgD
- C. IgG
- D. IgM
- E. IgE

**202. Restriction fragment length polymorphisms (RFLP) can be used in:**

- A. Analysis of inheritance of genetic trait within families
- B. Paternity testing
- C. Forensic identity testing
- D. B and C
- E. All of the above

**203. Figure 1 is a bone marrow aspirate from a patient with bleeding problem (inset, high-power review). What is the possible factor deficiency?**

- A. Factor II
- B. Factor V
- C. Factor VII
- D. Factor VIII
- E. Factor X



**Fig. 1**

**204. Figure 2 is a bone marrow aspirate from a patient with leukocytosis. What is the abnormal translocation?**

- A. RUNX1-RUNX1T1
- B. CBFB-MYH11
- C. PML-RARA
- D. MLLT3-MLL
- E. DEK-NUP214

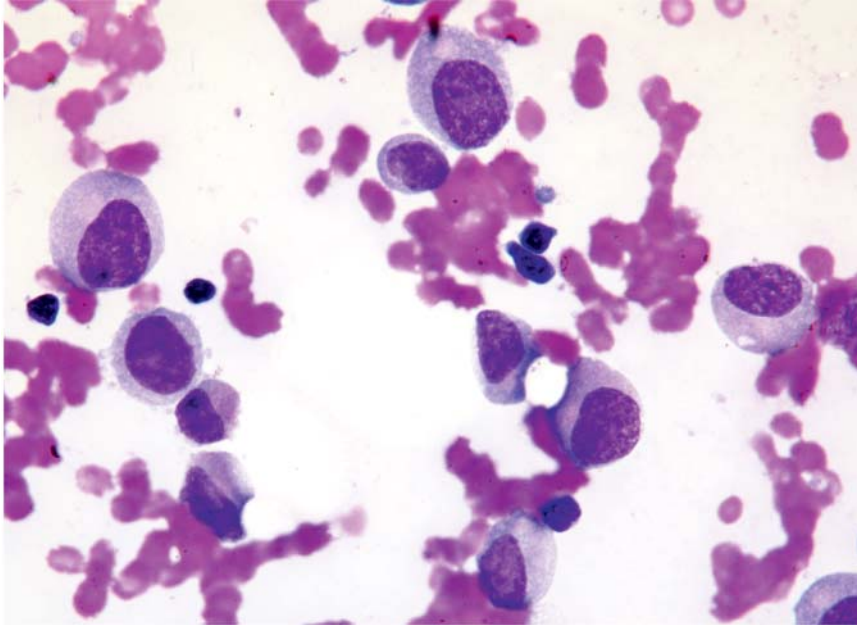


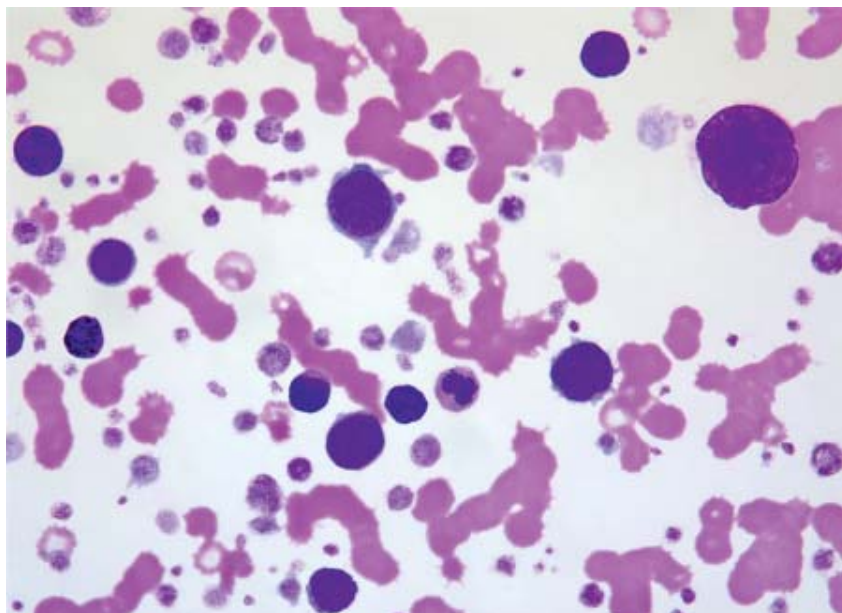
Fig. 2

**205. Figure 3 is a bone marrow aspirate from a patient with cytopenia and thrombocytosis. What is most likely diagnosis?**

- A. Myelodysplastic syndrome
- B. Polycythemia vera
- C. Essential thrombocytosis
- D. Acute megakaryoblastic leukemia
- E. Hairy cell leukemia

**206. Which of the following marker is present on these large cells (See Fig. 3)?**

- |         |         |
|---------|---------|
| A. CD2  | B. CD19 |
| C. TdT  | D. CD41 |
| E. CD68 |         |

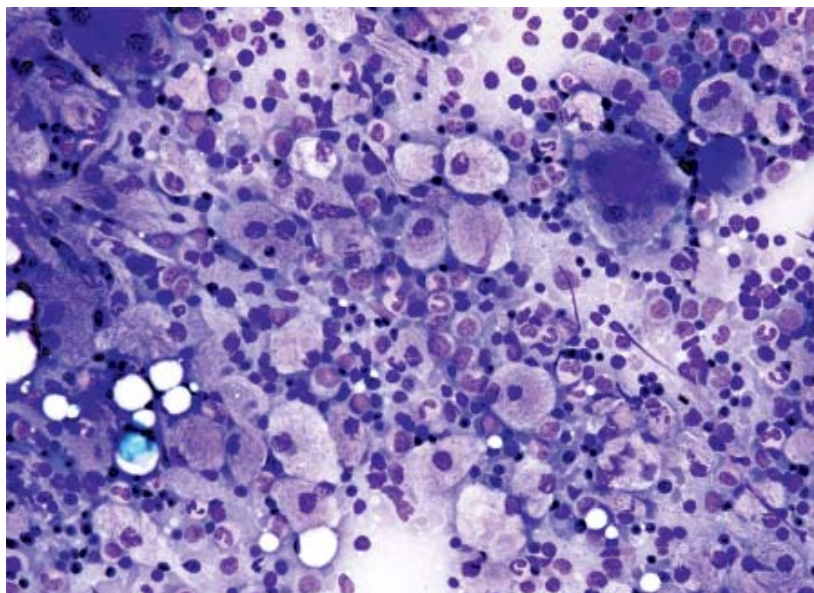


**Fig. 3**

**207. Figure 4 is bone marrow aspirate from a patient with splenomegaly.**

**Which of the following enzyme is defective?**

- |                                |                                     |
|--------------------------------|-------------------------------------|
| <b>A.</b> Glucocerebrosidase   | <b>B.</b> Alpha-galactosidase A     |
| <b>C.</b> Galactosylceramidase | <b>D.</b> Hexosaminidase-Alpha unit |
| <b>E.</b> Sphingomyelinase     |                                     |



**Fig. 4**



**208. Figure 5 is a spleen section from a 10-year-old boy who has an enlarged liver and spleen. What is most likely diagnosis?**

- A. Gaucher disease
- B. Niemann-Pick disease
- C. Histiocytic sarcoma
- D. Fabry disease
- E. Hepatosplenic T-cell lymphoma

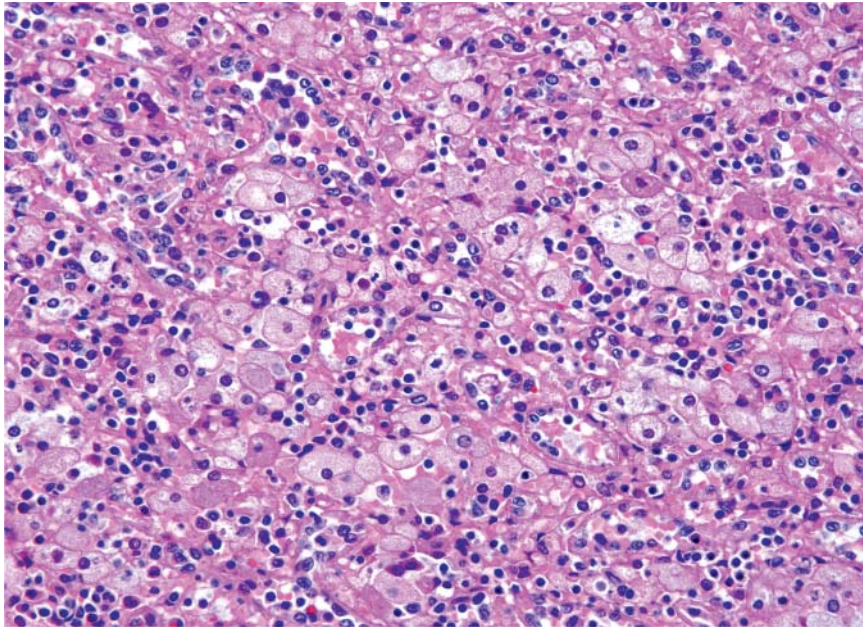


Fig. 5

**209. Figure 6 is bone marrow aspirate from a patient with anemia. What is the most likely diagnosis?**

- A. Acute leukemia
- B. Myelodysplastic syndrome
- C. Granuloma
- D. Metastatic adenocarcinoma
- E. Plasma cell neoplasm

**210. Figure 7 is bone marrow aspirate. What is the most likely diagnosis?**

- A. Metastatic adenocarcinoma
- B. Epithelioid granuloma
- C. Bone marrow aspirate artifact
- D. Osteoblasts
- E. Osteoclasts



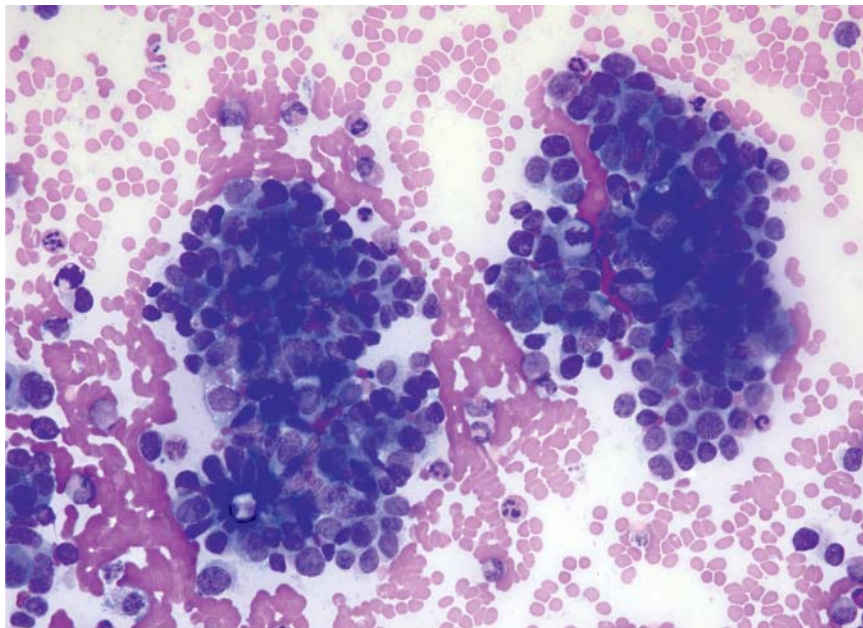


Fig. 6

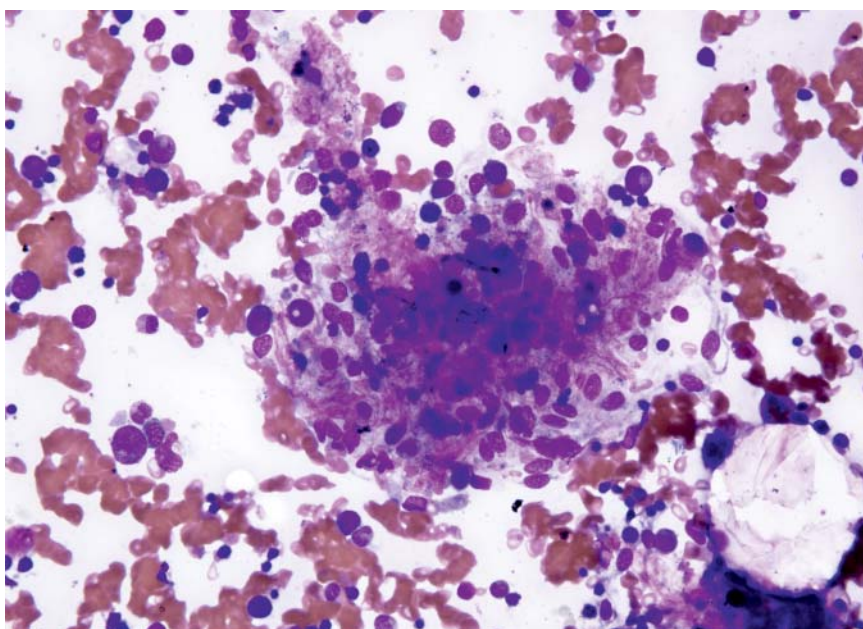


Fig. 7

**211. Figure 8 is bone marrow aspirate from a patient with anemia. What is the most likely diagnosis?**

- A. Histoplasmosis
- B. Metastatic melanoma
- C. Granuloma
- D. Hemosiderin loaded macrophages
- E. Preparation precipitation artifact

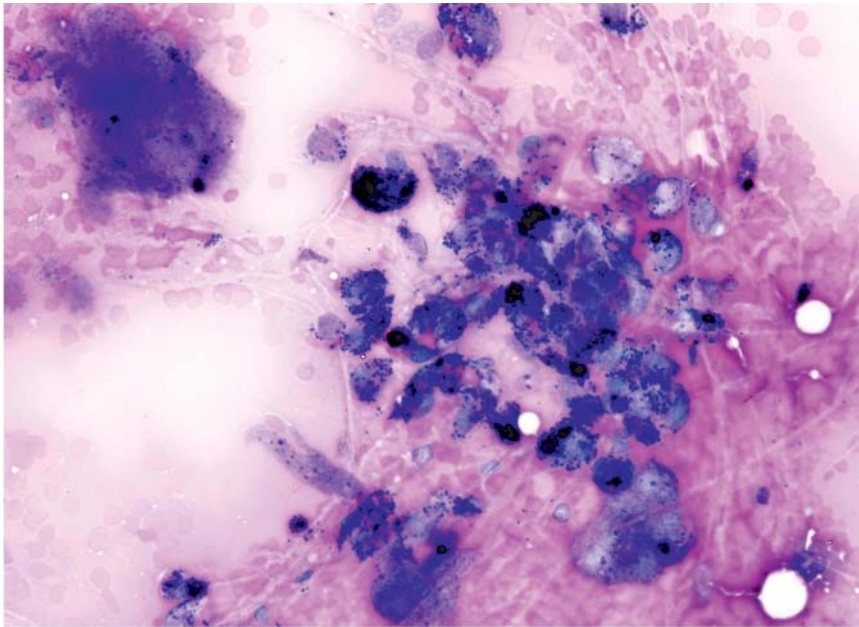


Fig. 8

**212. Figure 9 is a bone marrow aspirate from an AIDS patient (inset, high-power review). What is the most likely organism in the cytoplasm?**

- |                 |                 |
|-----------------|-----------------|
| A. Pneumocystis | B. Histoplasma  |
| C. Candida      | D. Pneumococcus |
| E. Giardia      |                 |

**213. Figure 10 is a peripheral blood smear. What is the most likely diagnosis?**

- |                 |                   |
|-----------------|-------------------|
| A. Pneumococcus | B. Histoplasma    |
| C. Candida      | D. <i>E. coli</i> |
| E. Pneumocystis |                   |

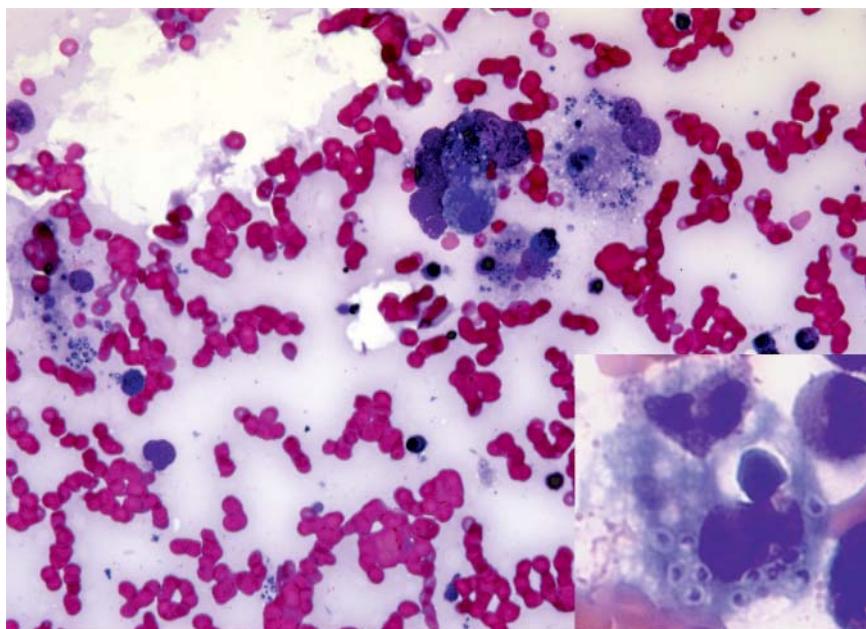


Fig. 9

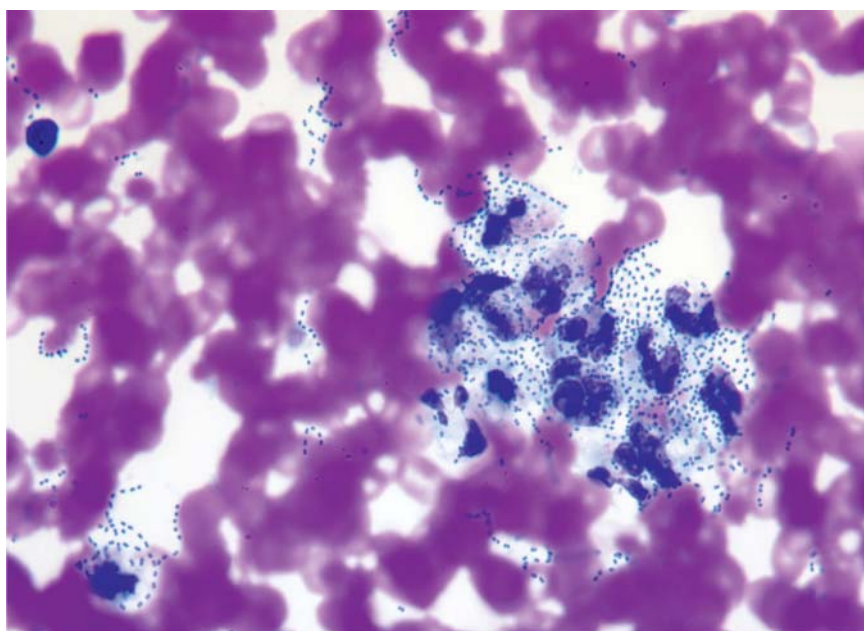


Fig. 10



**214. Figure 11 is a peripheral blood smear. What is the structure inside the red blood cells?**

- A. Cabot's ring
- B. *Plasmodium vivax*
- C. *Plasmodium falciparum*
- D. Heinz body
- E. Pappenheimer body

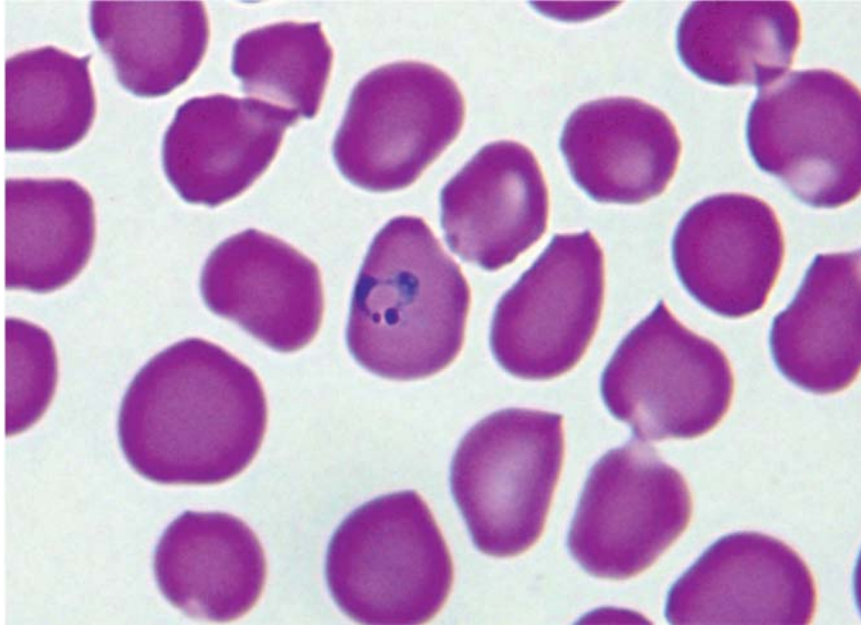


Fig. 11

**215. Figure 12 is a peripheral blood smear. What is the structure inside the red blood cells?**

- A. Cabot's ring
- B. *Plasmodium vivax*
- C. *Plasmodium falciparum*
- D. Heinz body
- E. Pappenheimer body

**216. Figure 13 is a peripheral blood smear. The most likely diagnosis is:**

- A. *Brugia malayi*
- B. *Schistosoma*
- C. *Leishmania*
- D. *Trichinella*
- E. Ehrlichiosis

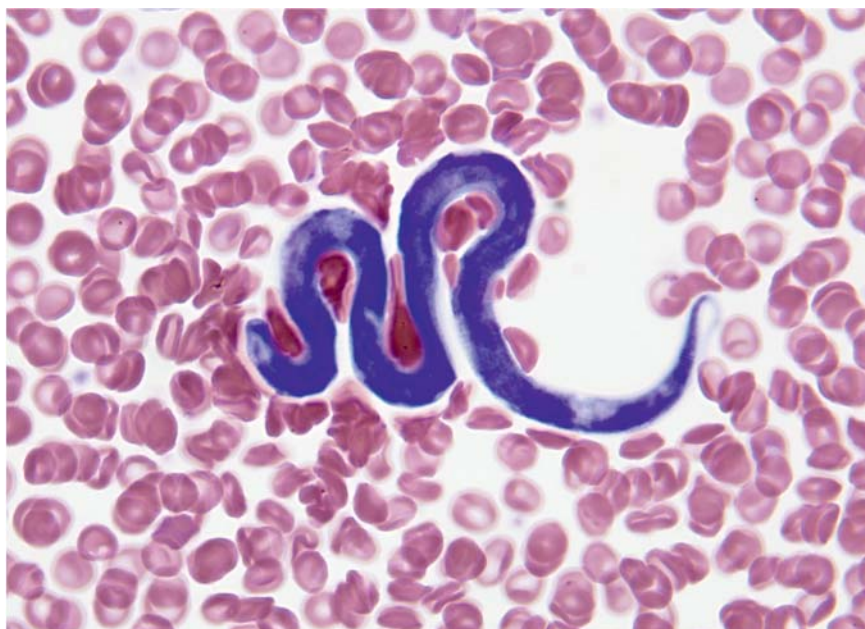
**217. Figure 14 is a peripheral blood smear. Which of the following description is correct?**

- A. This patient may have significant hyperviscosity
- B. This phenomenon is usually not associated with Hepatitis C virus infection



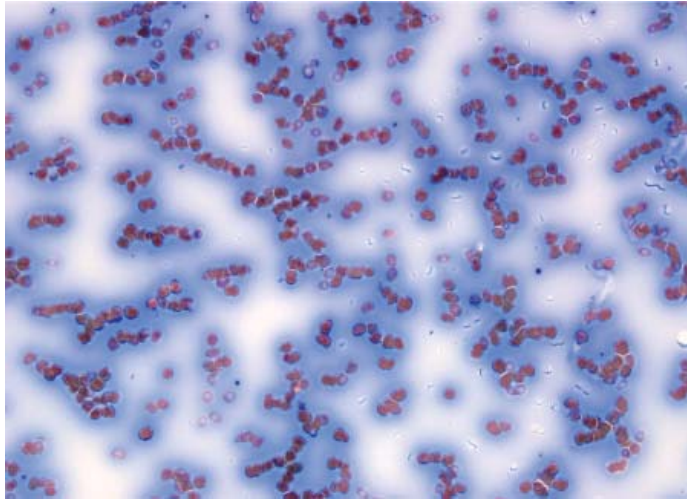


**Fig. 12**



**Fig. 13**

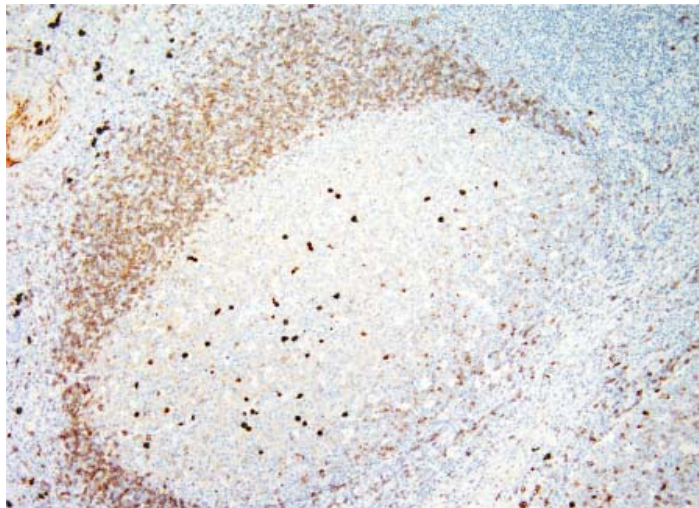
- C. It may cause clogging of automated instruments
- D. This patient may have acute renal failure
- E. All above



**Fig. 14**

**218. Figure 15 is a normal lymph node stained with an antibody, this antibody is:**

- A. IgA
- B. IgD
- C. IgE
- D. IgG
- E. IgM



**Fig. 15**

## *Answers*

**1. A**

**2. E**

**3. D**

**4. C**

Falsely elevated MCV may be present secondary to RBC clumping in cold hemagglutinin disease. Look for clumps on the peripheral blood smear, and warm the specimen to 37°C to avoid RBC clumping. Osmotic abnormalities can also cause falsely elevated MCV.

**5. C**

**6. C**

$$\text{Reticulocyte Production Index} = \frac{(\% \text{ reticulocytes} \times \text{Hct})}{45} \times \frac{1}{\text{correction factor}}$$

**7. B**

Directly measured: MCV (not affected), RBC (affected), Hgb (not affected)

Calculated: Hct (affected), MCH (affected, Hb/RBC), MCHC (affected, Hb/Hct)

**8. E**

**9. D**

**10. D**

EPO is predominantly produced in kidney, and to a lesser part in the liver. Hypoxia increases production of EPO and induces committed progenitor cells (CFU-E and BFU-E) to proliferate in the marrow. These committed progenitor cells differentiate into pronormoblasts by shortening generation time and promoting early release of reticulocytes into blood, therefore stimulate proliferation. Growth and differentiation, Erythropoietin gene is located on Chromosome 7.

Conditions associated with an increased EPO level include: erythroid hyperplasia, aplastic anemia, cerebellar hemangioma, renal cell carcinoma, hepatocellular carcinoma, renal cysts, hydronephrosis, and ovarian carcinoma.

Conditions associated with a decreased EPO level include: polycythemia vera, after transfusion, chronic renal disease, and hypothyroidism.

**11. A**

**12. C**

**13. C**

**14. B**

The neutrophil count in the blood is maintained in a normal steady state by the balance of granulopoiesis in the marrow, distribution of neutrophils between the marginated pool (in the microvasculature) and the circulating pool (in the blood), and the rate of egress from blood to tissues. Neutrophils have a short life span in the blood, with a half-disappearance time of approximately 7 hours. This process may be accelerated when inflammation is present, and highlights the need for a sustained rate of production to maintain a normal blood neutrophil count. At least four compartments are involved: marrow storage pool, circulating pool, marginated pool, and tissue pool.

**15. A**

**16. D**

Follicular dendritic cell: APC from follicle (S-100+, CD21+, CD1a-), follicular dendritic cell sarcoma may associated with Castleman's disease. Interdigitating reticulum cells: APC from paracortex (S-100+, CD21-, CD1a-).

Langerhans cells: APC from skin (S-100+, CD21-, CD1a-).

**17. B**

The primary stimuli for eosinophil production are interleukin (IL)-5, IL-3, and the granulocyte-macrophage colony-stimulating factor (GM-CSF). Eosinophil production and function are profoundly influenced by interleukin-5 (IL-5), other regulatory gene such as GATA are also associated with production of eosinophils.

**18. E**

**19. A**

**20. E**

Other fluorescent will stain reticulocytes include: thioflavin T, thiazole orange, auramine O, and oxazin 750. Congo red does not stain reticulocytes.

**21. D**

**22. B**



Immunophenotype of these small lymphocytes in thymoma are positive for CD1, CD2, CD3, CD5, CD7, and coexpress CD4/CD8. Some of them are also positive for TdT.

Stem cell thymocytes express CD2, CD7, and TdT.

Cortical thymocytes express CD2, CD7, TdT, CD1, CD5, cCD3, and show co-expression CD4/CD8.

Medullary thymocytes express CD2, CD3, CD5, CD7 and CD4 or CD8.

**23. E**

**24. A**

Alpha: 14q11

Beta: 7q34

Delta: 14q11

Gamma: 7p15

**25. E**

**26. A**

The order of Ig Heavy chain and light chain rearrangement is V-D-J, cytoplasmic mu, kappa and lambda; which all takes place in the bone marrow.

The earliest B-precursor cells in the bone marrow acquire cytoplasmic Ig heavy chain at the pre-B cell stage. The IgH and light chains fuse on the cell surface to form the intact Ig molecule.

IgH is located on chromosome 14q32 with 100 (V) variable regions and 15 to 20 (D) diversity regions and 6 (J) joining and the 9 (C) constant region genes that encode the particular Ig isotype: Cmu (IgM), Cdelta (IgD), Cgamma (IgG1-4), Calpha (IgA1-2) or Cepsilon (IgE). The heavy chain rearrangement begins with D combining with J, followed by combination with V region gene. The exons are deleted, the DNA is transcribed and undergoes RNA splicing with removal of intron material with splicing of V, D, J and Cmu (always Cmu first because it is the closest gene to the rearranged V-D-J segment) which now produces cytoplasmic IgH at the preB stage then gamma3,1 - alpha 1 - gamma2,4 - epsilon - alpha2 if nonproductive, due to termination or nonsense codon, the same process will begin on the other chromosome to make the same IgH.

Next, the Kappa light chain is made (no D regions and only one C region). If nonproductive, then process will begin on lambda gene (no

D region, 6 C region). The sequential kappa to lambda gene rearrangement process accounts for the 2:1 ratio of kappa to lambda.

**27. A**

The order of rearrangement is gamma, delta, beta and alpha. Alpha and Beta receptors are present on 95% of the circulating T-cells. Like the rearrangement of the immunoglobulin gene in developing B-cells, the TCR genes also undergo V(D)J rearrangement.

Location of Alpha, Beta, Gamma and Delta chain

Alpha: 14q11.2

Beta: 7q34

Delta: 14q11.2

Gamma: 7p15

**28. A**

**T-cell only deficiency**

DiGeorge syndrome is a nongenetic congenital syndrome of faulty development of third and fourth pharyngeal pouches with complete or incomplete (hypoplasia) absence of the thymus resulting in T-cell/cell mediated deficiencies. Infection by viruses/CMV, fungal, acid fast, Listeria and PCP are common. If there is complete absence of the thymus, patients usually die of overwhelming sepsis in first year of life. Other findings: neonatal tetany, hypothyroidism, severe congenital heart disease, dysmorphic facies hypertelorism, cleft lip, micrognathia, mental retardation. DiGeorge syndrome is multifactorial, most are sporadic, some congenital, some associated with monosomy 22.

Immunodeficiency with thymoma: thymic tumor, hypogammaglobulinemia, and half of them have abnormal T-lymphocytes.

**B- and T-cell deficiency**

Common Variable Immunodeficiency syndromes (CVTD)

Wiskott-Aldrich syndrome (X-linked)

Severe Combined Immunodeficiency (SCID): variable hereditary (X-linked most common)

Ataxia Telangiectasia (autosomal recessive)

Adenosine Deaminase Deficiency (autosomal recessive) is due to absence or malfunction of the purine degradation enzymes. Adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) results in accumulating purine metabolites (adenine, deoxy adenine, dATP) that are toxic to lymphocytes. The ADA deficiency causes combined deficiency (absent lymph node, thymus, tonsils, low T-cells and Igs).

The PNP deficiency enzyme causes only cell-mediated deficiency.

**B-cell deficiency only**

IgA deficiency.

X-linked agammaglobulinemia.

**29. D**

**30. D**

Yolk sac

**31. E**

**32. E**

**33. A**

Cabot ring is a red to purple ring forms in red cells. The significance is uncertain. It can be seen in megaloblastic anemia within reticulocytes and in an occasional, heavily stippled, late intermediate megaloblast. Their exact composition is questionable. Some investigators have suggested that Cabot rings originate from spindle material that was mishandled during abnormal mitosis.

**34. B**

Aplastic anemia does not cause splenic atrophy.

**35. A**

**36. E**

All of the following diseases are associated with low reticulocyte counts:

- Aplastic anemia
- Myelodysplasia
- Iron deficiency anemia
- Endocrinopathy (hypothyroidism, hypopituitarism)
- Renal disease
- Megaloblastic anemia
- Thalassemia.

**37. C**

1 mg iron/ml RBC or 0.5 mg/ml of whole blood

**38. C**

**39. D**

Glutamate formiminotransferase deficiency is a rare disorder, inherited in an autosomal recessive pattern: an inherited disorder that affects physical and mental development. Mutations in the FTCD gene result

in Glutamate formiminotransferase deficiency. There are two forms: mild and severe. The severe form may present as megaloblastic anemia, anemia responds to high dose folate.

Dihydrofolate reductase deficiency can also result in a megaloblastic anemia that responds to folate acid treatment.

Lesch-Nyhan syndrome can also present megaloblastic anemia due to HPRT/hypoxanthine-guanine phosphoribosyltransferase deficiency, does not respond to folate acid treatment.

**40. A**

**41. B**

Pitting function of spleen is removal of Heinz bodies (unstable Hemoglobins, G6PD, and drug induced oxidant injury) and forming bite cells. Pitting function of spleen can also remove malaria, Bartonella.

**42. C**

30% platelets are stored in the spleen, 60% in peripheral blood.

**43. E**

Whipple's disease usually has sinusoidal expansion and granulomatous lymphadenitis.

Follicular hyperplasia is also seen in post-vaccination, infectious mononucleosis, herpes, Dilantin treatment, CMV infection, and AILT.

**44. B**

**45. C**

Serum ferritin test is sensitive correlate with iron storage. However, it is also an acute phase reactant can be increased and mask iron deficiency anemia in patients with liver disease, malignancy, inflammatory disease, etc.

**46. A**

Transferrin level is decreased in thalassemia, chronic infections, malignancy, iron poisoning, hemochromatosis, hemolytic anemia, nephrotic syndrome, and malnutrition.

**47. B**

Serum iron men 55–160 mcg/dL, women 40–155 mcg/dL



**48. A**

Iron containing percentages in the body

Hemoglobin: 68%

Myoglobin: 3.3%

Ferritin: 12.7%

Hemosiderin: 11.7%

Transferrin: 0.17%

**49. C**

Conditions are associated with increased serum iron: Hyperferremia (following a high-iron meal, or with hemochromatosis and liver disease). Congenital hemochromatosis, thalassemia, inflammatory/immune acute hepatic necrosis, aplastic anemia, hemolytic anemia; metabolic/toxic excessive absorption (iron therapy, dietary excess), cirrhosis, pernicious anemia.

Conditions are associated with decreased serum iron: inadequate dietary intake, excessive blood loss (both with increased iron-binding capacity), or chronic inflammation (decreased iron-binding capacity), endocrine iron loss to the fetus during gestation, infectious/inflammatory/immune disease, rheumatoid arthritis (RA), SLE, mechanical/trauma intravascular hemolysis with hemoglobinuria (paroxysmal nocturnal hemoglobinuria, march hemoglobinuria, prosthetic heart valves), metabolic/toxic iron deficiency, repeated phlebotomy, diminished absorption (decreased ingestion, celiac disease, pica, postgastrectomy), neoplastic gastrointestinal cancers, loss of transferrin in nephrotic syndrome, psychosocial poverty, vascular intrapulmonary hemorrhage (e.g. idiopathic pulmonary hemosiderosis), chronic bleeding (e.g. menorrhagia, hematuria, peptic ulcer disease, gastritis, polyps, ulcerative colitis, colon carcinoma).

**50. B**

Idiopathic pulmonary hemosiderosis (IPH) is a rare, most often reported in children. Twenty percent of patients are adults and men are affected twice as frequently as women. Due to the recurrent episodes of diffuse alveolar hemorrhage, iron-deficiency anemia may develop. Pulmonary fibrosis with restrictive physiology and chronic obstructive lung disease has been reported as long-term complications from the diffuse alveolar hemorrhage. Because the disease is idiopathic, it is a diagnosis of exclusion established by tissue evaluation.

**51. D**

Splenectomy may not be necessary in very mild cases discovered late in adult life

**52. D**

Cytotoxic CD8+ T-cells and NK cells. Lymphocyte detected membrane antigen is a target Ag recognized by both NK and CD8+ T-cells, enabling them to destroy EBV-infected B-cells.

**53. A****54. A**

Positive IgM anti-VGA, high titer IgG anti-VGA and anti-EA indicate an acute EBV infection.

Positive IgG VGA, IgG EBNA, and negative IgM VGA indicate a remote infection.

**55. A**

Infectious mononucleosis: anti-i and sometimes anti-I

Paroxysmal cold hemoglobinuria: anti-P

Cold hemolytic agglutinin disease: anti-I

Mycoplasma pneumonia: anti-I

Post-transfusion purpura: anti-PI-A1

Rheumatoid arthritis: anti-Gm (antibody to the heavy chain of IgG)

Methyldopa: anti-e, sometimes anti-c

**56. A****57. E**

Hemophagocytic lymphohistiocytosis (HLH) is an unusual syndrome characterized by fever, splenomegaly, jaundice, and the pathologic finding of hemophagocytosis (phagocytosis by macrophages of erythrocytes, leukocytes, platelets, and their precursors) in bone marrow and other tissues. HLH may be associated with malignant, genetic, or autoimmune diseases and it is also prominently linked with Epstein-Barr virus (EBV) infection. Hyperproduction of cytokines, including interferon- $\gamma$  and tumor necrosis factor- $\alpha$ , by EBV-infected T lymphocytes may play a role in the pathogenesis of HLH. EBV-associated HLH may mimic T-cell lymphoma and is treated with cytotoxic chemotherapy. Hemophagocytic syndromes associated with nonviral pathogens often respond to treatment of the underlying infection.

**58. D**

Conditions that are associated with thrombocytopenia and large platelets are:

1. Bernard-Soulier
2. May-Hegglin
3. Gray platelet syndrome
4. Idiopathic thrombocytopenic purpura (ITP)
5. Montreal platelet syndrome

Wiskott-Aldrich syndrome is not associated large platelets but small platelets.

**59. A**

Response to ristocetin is absent in Bernard-Soulier syndrome and vWD except vWD 2B which has a hyperresponsiveness to low dose of ristocetin. Storage pool defect disorders include:

Chediak-Higashi syndrome

Thrombocytopenia with absent radii syndrome

Wiskott-Aldrich syndrome

Hermansky-Pudlak syndrome.

**60. E**

FEP is increased in iron deficiency, anemia of chronic disease, lead poison and some sideroblastic anemia, due to failure of Fe insertion into heme during erythropoiesis

**61. D**

**62. A**

An excellent way to identify hemolysis is a low haptoglobin. A low or absent haptoglobin, elevated plasma hemoglobin, hemoglobinuria and hemosiderinuria (occurs days later) indicate intravascular hemolysis. A peripheral smear is helpful too.

**63. C**

CMV infection usually results in pancytopenia and hypercellular bone marrow.

**64. E**

Thrombotic thrombocytopenic purpura (TTP) is not associated with giant platelets.

**65. B**

Factor X deficiency may be associated with amyloidosis, perform an abdominal fat pad or rectal biopsy to rule out amyloidosis.

**66. D**

**67. A**

The differential diagnosis includes vWD and XI deficiency. In vWD both factor VIII and vWF ristocetin activity are decreased. Factor VIII and IX deficiency are X-linked hereditary disorders. Deficiencies of factor XII, prekallikrein, high molecular weight kininogen do not cause bleeding disorder.

**68. A**

**69. C**

Lupus anticoagulant causes thrombosis but not bleeding. Answer B, D, E do not cause bleeding problems.

**70. B**

The Factor VIII macromolecular complex consists of vW Factor, HMW portion and Factor VIII:C.

Factor VIII:C (the low molecular weight part) is responsible for coagulant activity in conversion of X to Xa, hence the name Factor VIII: C (coagulation activity) and is measured by PTT and the PTT-derived Factor VIII assay

von Willebrand factor (vWF) is a central component of hemostasis that serves as a carrier for factor VIII and an adhesive link between platelets and the injured blood vessel wall. Abnormalities in vWF function result in von Willebrand disease (vWD), the most common inherited bleeding disorder in humans. vWF is also serves as a carrier for protein for VIII:C.

**71. E**

**72. E**

Also Stibophen.

**73. E**

**74. E**

**75. D**

**76. E**

Acute phase reactants

**77. E**

**78. E**



**79. B**

**80. E**

Congenital afibrinogenemia is exceedingly rare and produces mild to severe bleeding; the frequency of first-trimester miscarriage is increased among women with the disorder. The PT is more typically prolonged than the aPTT, and a functional fibrinogen assay shows reduced activity. Since no fibrinogen concentrate exists in the United States, treatment is with cryoprecipitate (preferred) or FFP and is aimed at increasing the plasma fibrinogen concentration to greater than 80 mg/dL.

Platelet adhesiveness and aggregation in congenital afibrinogenemia was studied in this paper: *Journal Annals of Hematology* 30(2), February, 1975 pages 87-100.

Result summary: Adrenalin-induced aggregation was absent whereas ADP-and collagen-induced aggregation was near normal or slightly decreased. Thrombofax aggregation was absent in citrated plasma. Ristocetin aggregation was normal in citrated plasma at the concentration of 1.5 mg per ml but it was absent at the lower concentration (1.0 mg per ml). Ristocetin aggregation was, on the other hand, absent in heparinized blood regardless of the concentration

**81. B**

Methyldopa as well as Fludarabine induces IgG antibody formation by absorbed onto RBC surface. Direct antiglobulin test (DAT) is positive.

**82. C**

Diamond-Blackfan syndrome or congenital red cell aplasia is one of the pure red cell aplasia disorders. The patients are associated with dysmorphic features including short stature, wide-set eyes, limb abnormality, triphalangeal thumbs and renal abnormality. Laboratory findings include increased serum iron, ferritin, folic acid, B<sub>12</sub>, EPO and HbF and decreased BFU-E. The differential diagnosis for pure red cell aplasia includes parvovirus infection (usually transient).

**83. D**

>65% of hereditary spherocytosis is autosomal dominant pattern.

**84. E**

**85. E**

**86. E**

A wide variety of medications and diagnoses, including malignant neoplasms, cardiovascular disease, hepatobiliary disease, and alcoholism,

are associated with stomatocytosis. Both vinblastine and chlorpromazine produce a time- and concentration-dependent stomatocytic shape change.

**87. E**

**88. A**

Alpha thalassemia and Sick cell trait: Hgb S <35%. Hgb S is less than 35% often indicates coexistent alpha thalassemia, which also shows microcytosis.

Sickle cell disease: Hgb A 0, S > 80%, F 1-20% (evenly distributed in RBCs), A2 2-4.5%.

Sickle cell trait: Hgb A 50-65%, S 35-45%, A2 normal to slight increased.

Sickle cell and thalassemia Beta<sup>0</sup>: A 0, S 75-90%, F 5-20%, A2 >4.5%.

Sickle cell and thalassemia Beta<sup>+</sup>: Hgb A 5-30, S>50%, F 1-20%, A2>4.5%.

**89. C**

**90. A**

**91. E**

**92. D**

The diagnosis of pyruvate kinase deficiency is occasionally suggested by the presence of spiculated spheroid cells on the peripheral blood smear, usually increased post-splenectomy. A positive Coombs test indicates an autoimmune related hemolysis.

The diagnosis depends on the demonstration of low levels of pyruvate kinase activity in red cells. More recently, DNA analysis using polymerase chain reaction or single-strand conformation polymorphism are available to confirm the diagnosis and to identify the carrier state. The pyruvate kinase activity is usually normal in white cells, platelets, and other tissues in the patient with pyruvate kinase deficiency. Because of the chronic hemolysis, a bone marrow examination reveals erythroid hyperplasia and active marrow.

**93. E**

50% Alpha thalassemia trait (-A/-A, -A/-A, AA/-A, AA/-A).

**94. D**

Sickle cell disease and thalassemia Beta<sup>+</sup>. Hemoglobin electrophoresis of more Hbs than HbA, increased A2 and a small amount of HbF is virtually diagnostic of HbS- Beta<sup>+</sup> thalassemia in an untransfused patient. In the case of sickle cell disease and thalassemia Beta<sup>0</sup> HbS is 100% and HbA is 0%.

**95. A**

Type 1: normal pattern, but decreased in quantity

Type 2A: loss of intermediate and high molecular weight multimer

Type 2B: loss of high molecular weight multimer

Type 3: total absent

**96. B**

**97. A**

**98. B**

**99. B**

Increased ESR is associated with the following conditions:

increased fibrinogen or alpha<sub>2</sub>, beta and gamma globulins (promotes RBC rouleaux by decreasing zeta potential), too high anticoagulant concentration or use of heparin, tilting the tube, increasing temperature, cholesterol, rouleaux, macrocytics anemia, pregnancy, monoclonal gammopathy, hypergammaglobulinemia, inflammatory diseases, autoimmune/collagen vascular disease, neoplastic diseases, temporal arteritis and polymyalgia rheumatica. ESR is also increased with age. Decrease ESR is associated with the following conditions: hypofibrinogenemia, polycythemia, sickle cell anemia, hereditary spherocytosis, and congestive heart failure.

**100. A**

**101. A**

X-linked: VIII and IX, Wiskott-Aldrich syndrome.

Autosomal dominant: vWD, platelet-type vWD, dysfibrinogenemia, antithrombin III, protein C, protein S, Activated protein C resistance/ Factor V Leiden.

Autosomal recessive: (all the rest) II, V, VII, X, XI, XIII, alpha<sub>2</sub>-plasmin, Glanzmann's thrombasthenia, Bernard-Soulier's disease, Dense granule deficiency (Hermansky-Pudlak, Chediak-Higashi, TAR syndrome).

**102. D**

**103. B**

It is an unstable hemoglobin.

**104. D**

**105. C**

V, VIII and fibrinogen are at normal levels at birth.

**106. B**

Factor VII deficiency. Barium sulfate absorbed plasma contains fibrinogen, V, VIII, XI, XII and XIII (Vitamin K-dependant factors II, VII, IX and X were absorbed).

**107. B**

Factor VII deficiency.

The Russell's viper venom time is used to assess for abnormalities in the extrinsic system or prolonged PT. It activates Factor X, bypassing the deficient Factor VII, so a normal Russell's viper venom time with an abnormal PT time supports factor VII deficiency.

**108. E**

Hemoglobin Bart's can also give a positive sickling test.

**109. A**

**110. D**

Lecithin-cholesterol acyltransferase (LCAT) is an enzyme bound to high-density lipoproteins (HDLs) and low-density lipoproteins in the plasma. LCAT catalyzes the formation of cholesterol esters in lipoproteins. The two familial forms of LCAT deficiency are termed familial LCAT deficiency (complete LCAT deficiency) and fish eye disease (partial LCAT deficiency).

The clinical manifestations of LCAT deficiency are probably due to a defect in LCAT-mediated cholesterol ester formation and, therefore, accumulation of unesterified cholesterol in certain tissues, such as the cornea, kidneys, and erythrocytes. Fish eye disease is characterized by partial reduction of LCAT and only manifests with progressive corneal opacification.

The major morbidity and mortality of familial LCAT deficiency is related to renal failure.

In fish eye disease, the major morbidity is corneal opacities causing visual impairment.

**111. D**

RT and TT are prolonged in dysfibrinogenemia/hypofibrinogenemia, and in disseminated intravascular coagulation, due to hypofibrinogenemia and the interference of fibrin degradation products with fibrin polymerization.

RT normal, but TT prolonged in heparin, the addition of protamine sulfate will correct the TT due to heparin.



Paraproteins, uremia, some lupus anticoagulants and antibody to thrombin will also prolong the TT.

**112. E**

The Wiskott-Aldrich syndrome (WAS) is characterized by eczema, thrombocytopenia, and recurrent infections. The gene for Wiskott-Aldrich syndrome protein (WASP) is located on Xp11.22, and the syndrome is inherited as X-linked recessive. WAS patients have a specific inability to respond normally to polysaccharide antigens. Serum IgM levels usually are low, whereas IgG and IgA levels can be normal or elevated. With increasing age, patients become lymphopenic and have severely impaired cell-mediated immunity. Because of their immunodeficiency, affected boys rarely survive beyond the first decade of life because of overwhelming infections with gram-positive and gram-negative bacteria, viruses, and fungi. WAS patients may also suffer from hemorrhage and lymphoreticular malignancies.

**113. E**

These are autosomal recessive disorders with hypersensitivity to one or more DNA damaging agents and a predisposition to cancer

**114. E**

Low-birth-weight infants are born with low serum and tissue concentrations of vitamin E. If these infants are fed a diet rich in polyunsaturated fatty acids and inadequate in vitamin E, a hemolytic anemia will develop by 4 to 6 weeks of age. The anemia often is associated with an increase in reticulocytes, small dense poikilocytes, and keratocytes (bitten cells) seen on peripheral blood smear, thrombocytosis, and edema of the dorsum of the feet and pretibial area. Current infant formulas have helped in eliminating vitamin E deficiency in preterm infants.

**115. D**

Factor deficiencies may cause thrombosis:

Antithrombin III, protein C, protein S, APC resistance, XII (Hageman), prekallikrein, HMW Kininogen, Heparin cofactor II (heparin cofactor II is an inhibitor of thrombin), plasminogen, plasminogen activator.

Thrombosis may also be associated with elevated plasminogen activator inhibitor, 10% of dysfibrinogenemias, lupus anticoagulants, anticardiolipin antibody, and cystathionine beta-synthetase deficiency.

**116. B**

Factor VII 4-6 hours

**117. E**

Coagulation factors that may become deficient in nephrotic syndrome include: antithrombin III, XII, IX, and prekallikrein. Deep vein thrombosis and pulmonary emboli are well recognized risks in nephrotic syndrome patients.

**118. E****119. C**

The following coagulation factors are increased during pregnancy: VIII, vWF, fibrinogen and Vitamin K dependant factors VII, IX, X (but not II). Impaired fibrinolytic activity and increased fibrinolytic inhibitors during pregnancy also contribute to the increased risk of deep vein thrombosis.

**120. D****121. D****122. A**

Lack of Alpha granules, result in deficiency in platelet factor 4, beta thromboglobulin, fibrinogen, and platelet derived growth factor/PDGF, and thrombospondin.

Autosomal dominant, thrombocytopenia, bleeding starting in infancy, morphologic abnormality in platelets and megakaryocytes (vacuolated/ honey-combed on EM and gray hypogranular platelets on PB).

**123. C**

Transcobalamin III does not significantly bind B<sub>12</sub>, found in special granules of neutrophils. Transcobalamin III is made in fibroblasts, macrophages, enterocytes, renal cells, liver, gastric mucosa and endothelium.

Transcobalamin I is a passive reservoir in equilibrium with liver storage, 70-90% saturated, synthesis in granulocytes, increased in CML due to break down. Transcobalamin I deficiency will not result in megaloblastic anemia; however, the level of cobalamin is decreased.

Transcobalamin II binds 90% of newly absorbed B<sub>12</sub>, it is the chief transport protein. Transcobalamin II synthesis in liver, macrophages, endothelium and ileum, 5% saturated. Transcobalamin II deficiency results in megaloblastic anemia, but serum cobalamin level is normal.

**124. E**

Hexokinase  
 Pyruvate kinase  
 Aldolase  
 Glucosephosphate isomerase  
 Phosphofructokinase  
 Triosephosphate isomerase

Phosphoglycerate kinase with the exception of hexokinase and pyruvate kinase are all additionally associated with neuromuscular impairment. Therefore, all kinases, isomerases and aldolases are associated with hemolysis but not the dehydrogenases, mutases, enolases.

**125. E**

Based on an old case report, deficiency of coagulation factors VII and XII in a patient with Hodgkin's disease. Arch Intern Med. 1977 Nov;137(11):1633-5.

VII and XII deficiency present in a patient with Hodgkin lymphoma. After treatment, level usually return to normal.

**126. D**

Platelet alpha granules contain factor V (not dense granules).

**127. D**

The thrombocytopenia and absent radii (TAR) syndrome is a rare disease, first identified in 1959, that occurs with an approximate frequency of one in 500,000 to 1,000,000 births. The inheritance pattern of TAR is unknown. The disease is characterized by the absence of radii and thrombocytopenia. TAR has low platelet count, usually in 15-50,000 range with a paucity of bone marrow megakaryocytes, skin bruising and a peripheral left-shifted leukocytosis. Thrombocytopenia may be severe early in life but tends to improve with age. Thus, given a strong clinical suspicion, the diagnosis should not be excluded based on one isolated normal cell count. The etiology of the thrombocytopenia is unknown; probably related to the defect directly involves the megakaryocytes, causing an early arrest in megakaryopoiesis. Bone marrow cellularity is normal or increased. Megakaryocytes are low in number, or absent, or appear immature. Affected patients can be managed with platelet transfusions and supportive treatment. Treatment usually is required only at early ages when the thrombocytopenia is more severe. Death is most commonly caused by bleeding in very young patients. Death related to thrombocytopenia occurring after age 14 months is rare.

**128. C**

Platelets may become dysfunctional, prolonging the bleeding time as blood circulates through a pump oxygenator during cardiopulmonary bypass. The mechanism appears to be activation of fibrinolysis on the platelet surface with resultant loss of the glycoprotein Ib-IX binding site for von Willebrand's factor. Cardiac bypass surgery patients are given protamine sulfate to reverse the anticoagulant effect of the heparin given to prevent clotting while the patient is on the heart-lung bypass machine. The excess protamine sulfate in the patient's plasma can impact the results of coagulation screen tests. A clue that this may be true is a shorter than reference range TT result. The heparin/protamine ratio of 1:1.5 is optimal for reversal of heparin-induced platelet dysfunction, and higher heparin/protamine ratios have significant antiplatelet effects secondary to excess protamine that affect aPTT and PT result (Anesthesia & Analgesia July 2001 vol. 93 no. 1: 20-27). Heparin rebound is defined as 'the reactivation of heparin effect that occurs from 5 minutes to 5 hours after neutralization with protamine sulfate in cardiopulmonary bypass.

**129. E**

Drugs that may cause hemolysis in G6PD deficiency:

Acetanilid, Methylene blue, Naphthalene, Niridazole, Pamaquine, Pentaquine, Phenylhydrazine, Sulfacetamide, Sulfanilamide, Sulfamethoxazole, Sulfapyridine, Thiazolsulfone, Toluidine blue, Trinitrotoluene (TNT), Primaquine (antimalarials), Sulfonamides, Nitrofurantoin, Nalidixic acid.

**130. A**

Vitamin E (tocopherol), a fat soluble vitamin and an antioxidant which prevents damage to lung, tissue, red blood cells and neurons. Vitamin E is synthesized by plants and found in high concentration in vegetable oils, egg yolks, liver and milk. Vitamin E deficiency in premature infants is characterized by edema, hemolytic anemia, thrombocytosis, erythematous skin rash and papular skin lesions. Deficiency in adults is rare.

**131. A**

HPP is a rare cause of anemia with erythrocyte morphology similar to that seen in patients suffering severe burns. The erythrocytes from these patients also exhibited increased thermal sensitivity. A strong relationship exists between hereditary elliptocytosis (HE) and HPP.



Approximately one third of parents or siblings of patients with HPP have typical HE, and many of these family members share identical mutations in erythrocyte spectrin. In addition, many patients with HPP proceed to develop typical mild to moderate HE. Patients with HPP tend to experience severe hemolysis and anemia in infancy that gradually improves but then evolves toward typical hemolytic HE later in life. It is common in individuals of African and Mediterranean descent, presumably because elliptocytes confer some resistance to malaria. The principal defect in HE and HPP is erythrocytes membrane proteins abnormalities including spectrin, protein 4.1, and GPC. The majority of defects occur in spectrin. Structural and functional defects of protein 4.1 lead to disruption of spectrin–actin attachment to the membrane via GPC, causing changes in cell shape and membrane stability similar to those found in abnormalities of spectrin.

**132. E**

Enzyme deficiencies that are associated with decreased 2,3 DPG level  
 2,3 DPG mutase deficiency  
 2,3 DPG phosphatase deficiency  
 Glucose phosphate isomerase deficiency  
 Phosphofructokinase deficiency  
 Enzyme deficiencies that are associated with an increased 2,3 DPG level  
 Pyruvate kinase deficiency  
 Phosphoglycerate kinase deficiency.

**133. B**

**134. C**

Diseases and their associated enzyme deficiency:  
 Krabbe disease - Galactosylceramidase  
 Fabry disease - Alpha-Galactosidase A  
 Gaucher's disease - Glucocerebrosidase  
 Niemann-Pick disease - Sphingomyelinase  
 Pompe disease (type 2) - Alpha 1, 4-Glucosidase

**135. A**

Autosomal recessive inheritance pattern

**136. E**

**137. E**

**138. A**

**139. E**

Hemoglobin G-Philadelphia is an abnormal alpha chain disorder that result of asparagine to lysine substitution at 68th position of the alpha globin chain. It is common in African-American population and has no clinical or hematological effects.

On electrophoresis, Hemoglobin G-Philadelphia migrates slower than A2 close to origin and even slower than carbonic anhydrase. The present of Hb-G2 band on the alkaline electrophoresis (usually difficult to visualize) or isoelectric focusing is helpful for the diagnosis.

**140. B**

D, G, Lepore will migrate in the Hb S zone.

**141. E**

Please see answer 106

**142. E**

The aged normal serum contains factors VII, IX, X, XI and XII.

**143. D****144. A****145. C**

Features of JMML

1. Monosomy 7 (also called monosomy 7 syndrome)
2. No Ph chromosome
3. Marked male predominance
4. Skin rash, extramedullary infiltrates and recurrent infections
5. Leukocytosis with neutrophilia and monocytosis and hepato-splenomegaly
6. Anemia with NRBC and thrombocytopenia
7. Dysplasia with dysplastic monocytes
8. Increased Hb F
9. High incidence of evolution to acute leukemia
10. -5, -5q are uncommon
11. 10-15% of the patients are associated with NF-1

**146. B**

A leukoerythroblastic reaction may be associated with all of the following conditions:

1. Marrow infiltration (leukemia, carcinoma, lymphoma)
2. Chronic myeloid proliferative disorders

3. Severe hemolysis
4. Osteopetrosis
5. Hypoxia
6. Infection.

**147. E**

**148. C**

**149. D**

**150. C**

**151. B**

**152. E**

The causes of absolute polycythemia secondary to EPO production including:

hepatocellular carcinoma, renal cell carcinoma, cerebellar hemangioma, lung tumors, and tumors of uterus, breast, thymus, smokers, methemoglobinemia, renal disease, cysts, renal artery stenosis, hydronephrosis, masses impinging on the kidneys, high O<sub>2</sub> affinity hemoglobinopathies, 2,3 DPG deficiency, high altitude, chronic hypoxic (disease of cardiac and lung).

**153. D**

Acute myelomonocytic leukemia (FAB M4), and acute monoblastic/monocytic leukemia (FAB M5) have high risk of CNS and extramedullary involvement (including skin).

**154. B**

**155. A**

**156. E**

**157. E**

BCR-ABL1 210Kb protein: 8.5 Kb mRNA, CML, ALL

BCR-ABL1 190 Kb protein: 7.0 Kb mRNA frequently associated with ALL, however, a small amount of p190 can be detect in >90% of p210 CML. CML with p190 fusion protein has an increased numbers of monocytes, it can be confused with CMML

BCR-ABL1 230Kb fusion protein: CML with this fusion protein may demonstrate prominent neutrophilic maturation and/or conspicuous thrombocytosis.

**158. A**

**159. D**

**160. C**

**161. C**

**162. B**

**163. E**

Precursor B-ALL is cytoplasmic mu negative

**164. B**

**165. C**

The typical immunophenotype of Hairy cell leukemia is positive for SIg (M+/-, D, G or A), CD19, CD20, CD79a, CD11c (strong), CD22, CD25 (strong), CD103, FMC7 and negative for CD5, CD10, CD23. CD103 is the most helpful marker of all because CD11c, CD22, CD25, FMC7 and even TRAP can be present in other disorders, therefore, the diagnosis is based on strong expression of these markers in conjunction with characteristic morphology.

**166. A**

Typical immunophenotype of mantle cell lymphoma is positive for SIg, usually IgD, Lambda>kappa, CD 19, CD20, CD79, CD5 (distinguishes from FCL and marginal zone B-cell lymphoma), CD43, CD10-/-, but negative for CD23 (distinguished from SLL) and CD11c. t(11;14) involves bcl-1 locus on 11 and Ig heavy chain on 14 (over-expression of PRAD1 gene which encodes for cyclin D-1).

**167. C**

The immunophenotype of adult T-cell lymphoma/leukemia is usually: CD2+, CD3+, CD5+, and CD7-. Most cases are CD4+/CD8- (rare case are CD4-/CD8+ or CD4+/CD8+). Strong expression of CD25 is present in majority of cases.

**168. C**

	<b>EATL</b>	<b>Type II EATL</b>
Frequency	80-90%	10-20%
Morphology	Pleomorphic	Monomorphic
CD8	Mostly negative (80%)	Mostly positive (80%)
CD56	Mostly negative (>90%)	Mostly positive (>90%)
+9q31.3 or -16q12.1	Common	Common
+1q32.2-q41 or +5q34-q35.2	Common	Uncommon
+8q24 (MYC)	Uncommon	Common



**169. C**

The most common translocation of Burkitt's lymphoma is t(8;14), cMYC (8q24) to Ig heavy chain (14q32). Less commonly cMYC (8q24) to Kappa light chain genes on chromosome 2 to form t(2;8) or to Lambda light chain gene on chromosome 22 to form t(8;22).

Immunophenotype of Burkitt's lymphoma: SIgM+, CD19+, CD20+, CD22+, 79a+, CD10+, CD5-, CD23-.

EBV positive in most African/endemic cases and 25-40% of HIV-associated cases and less common in non-African, nonimmune deficiency related cases.

**170. B**

t(14;18), the bcl-2 gene (18q21) is translocated to the heavy chain gene on 14q32. bcl-2 prevents programmed cell death by extending survival.

**171. D**

Precursor B-ALL expresses various combinations of B-lymphocyte associated antigens CD19, CD22, CD79a, CD24, and CD10 (CD10 usually negative in infant and in 25% of adult B-ALL).

CD20 is highly specific for precursor B-ALL, but usually absent or dim in many cases. Surface immunoglobulin (sIg) is absent.

Cytoplasmic immunoglobulin m chain (cIg) is present in about 20% of the Precursor B-ALL.

There is no light chain expression.

Other non-specific antigens include: TdT (90%), CD34 (75%), HLA-DR (98%), CD38 (98%) and CD45 (70%).

**172. D**

**173. E**

**174. D**

**175. B**

**176. E**

PTGCs are usually found in follicular hyperplasia, and nodular lymphocyte predominant Hodgkin lymphoma.

**177. B**

**178. C**

NK large granular cell leukemia (NK-LGL) is more aggressive than T-cell large granular cell leukemia. It is not associated with autoimmune disease. Morphology shows acute lymphoblastic-like cells with granular

cytoplasm. NK-LGL may be associated with a high WBC count, there is more extensive involvement of bone marrow and widespread infiltrates of organs. NK-LGL is associated with a short median survival. Immunophenotype positive for CD2, CD7(+/-), CD8(+/-), CD16, and CD57 and negative for CD3, CD4. No gene rearrangements, germline chromosomes.

T-cell large granular cell leukemia (T-LGL).

Most of them have an indolent course, frequent association with rheumatoid arthritis and autoimmune disease polyclonal gammopathy/autoimmune antibody, neutropenia and splenomegaly may present. Immunophenotype is positive for CD2, CD3, CD7, CD8, CD56, and CD57 and negative for CD4. T-cell gene rearrangement is clonal.

**179. A**

Sézary syndrome: CD2, CD3, CD4, CD5 positive; CD7 absent or weak; and CD8 negative.

Adult T-cell leukemia: CD2, CD3, CD4, CD5, CD25 positive, CD4, CD8 positive or CD4 and CD8 negative, CD7 absent or weak.

Hairy cell leukemia: Strong sIg, CD19, CD20, CD22, CD11c positive, variable CD25 and CD103.

Follicular cell lymphoma: Strong sIg, CD19, CD20, CD22, and CD10 positive.

T-cell prolymphocytic leukemia: CD2, CD5, CD4, CD7 positive, CD8 negative involving 14q11 common.

**180. C**

**181. B**

Histological features of angioimmunoblast T-cell lymphoma (AITL) includes: atrophic germinal centers, partially obliterated sinuses or open dilated sinuses, vascular proliferation, plasma cells, epithelioid histiocytes, clusters of clear cells, and expanded follicular dendritic cell clusters around vessels. EBV infection is common due to T-cell dysfunction. Patients usually have systemic disease with generalized lymphadenopathy, fever, weight loss, skin rash.

Both T and B gene rearrangement may be positive. The B-cell clonal gene rearrangement is due to a B-cell clonal proliferation caused by EBV infection.

**182. E**

The detection of platelet peroxidase in perinuclear cisternae and endoplasmic reticulum by ultrastructural methods may be the earliest

method to identify AML-M7, but immunophenotypic and flow cytometry methods are the preferred. Smear techniques are preferred over flow because of false positive reactions from platelets adhering to blasts. CD41(GpIIb/IIIa), most valuable, deficiency in Glanzmann's thrombasthenia.

CD42 (GpIb) deficiency in Bernard-Soulier syndrome CD61 (IIIa) AML-M7 is associated with trisomy 21 (most common AML in Down's syndrome) and with t(1;22).

**183. B**

Reed-Sternberg cells of classic Hodgkin lymphoma are usually CD15+/-, CD20-/+ , CD30+, CD45-, Fascin+.

The large cells of nodular lymphocyte predominant Hodgkin lymphoma are usually CD15-, CD20+, CD30-. Approximately 2-5% of nodular lymphocyte predominant Hodgkin lymphoma progress to diffuse large B-cell lymphoma. Unlike classic Hodgkin lymphoma's bimodal age distribution, nodular lymphocyte predominant Hodgkin lymphoma has a single age distribution.

**184. D**

The lineage of Reed-Sternberg cells is pre-apoptotic germinal center B-cell. The L&H cell of nodular lymphocyte predominant Hodgkin lymphoma is antigen-selected mutating germinal center B-cell.

**185. A**

**186. B**

**187. E**

**188. A**

**189. B**

Rai staging system

0: Lymphocytosis in blood and BM only, median survival 120 months

I: Lymphocytosis and LAD, median survival 95 months

II: Lymphocytosis and HSM, +/-LAD, median survival 72 months

III: Lymphocytosis and anemia (< 10 mg/dl), median survival 30 months

IV: Lymphocytosis and thrombocytopenia (<100,000), median survival 30 months

Binet staging system

A. Hb>10 mg/dl, platelet > 100,000, <3 anatomic sites involved, median survival 120 months

B. Hb >10 mg/dl, platelet > 100,000, >3 anatomic sites involved, median survival 61 months

C. Hb <10 mg/dl, platelet < 100,000, or both, median survival 32 months

**190. A**

B-ALL has a good prognosis in children, but is less favorable in adults, < 1 year-old or >10 year-old, high WBC count, present minimal residual disease have adverse prognosis. In children and adults, t(9;22) translocation indicates a poor prognosis.

**191. E**

**192. C**

Lutzner et al (1973) have described a small cell variant of Sézary cells, and these are being referred to as Lutzner cells.

**193. C**

**194. B**

**195. E**

**196. E**

**197. A**

The head and neck region, most commonly in the nasopharynx/nasal cavity/paranasal cavities, with spread to bone marrow in 40% of cases, most often a single osteolytic lesion. 15% of the cases have multiple bone deposits or marrow plasmacytosis. Prognosis is excellent if localized, but similar to multiple myeloma if spread to bone.

**198. D**

Unmutated IgVH, High percentage of ZAP70 and CD30 are poor prognostic markers of CLL/SLL.

Poor prognosis markers: severe anemia, poor performance rating, renal failure, high beta2-microglobulin.

Less important: hypoalbuminemia, hypercalcemia, high monoclonal component and expression of CD 10 on plasma cells.

Genetic abnormalities are also associated with prognosis.

**199. A**

**200. D**

IgD



**201. B**

IgD

**202. E**

Restriction fragment length polymorphism is a type of DNA-based allelic variation in which different alleles at a single locus are recognized and followed through pedigrees based on the size of a restriction fragment. The locus is defined by the segment of DNA that gives rise to the restriction fragment; the different alleles are generally (not always) caused by a single change in DNA sequence that creates or abolishes a site of restriction enzyme cleavage.

**203. E**

The bone marrow aspirate showed diffuse deposits of amyloid stained by Giemsa. The amyloid appear as amorphous, nebulous navy blue material. Amyloidosis is associated with factor X deficiency.

**204. C**

Acute promyelocytic leukemia, microgranular variant.

RUNX1-RUNX1T1 – t(8;21)

CBFB-MYH11 – inv16 or t(16;16)

PML-RARA – t(15;17)

MLLT3-MLL – t(9;11)

DEK-NUP214 – t(6;9)

**205. D**

Acute megakaryoblastic leukemia.

**206. D**

CD41 and CD61 are most specific for acute megakaryoblastic leukemia.

**207. A**

### **Gaucher Disease**

Gaucher disease is an autosomal recessive disorder that results from defective activity of glucocerebrosidase (also known as acid  $\beta$ -glucosidase); >250 mutations have been described. The type of Gaucher disease is classified based on the absence or presence and progression of neuronopathic involvement.

Type 1 is a nonneuronopathic disease that can present in childhood to adulthood with slowly to rapidly progressive visceral disease.

Type 2 is a rare, severe CNS disease that leads to death by 2 years of age.

Type 3 has highly variable manifestations in the CNS and viscera and is common among individuals of Swedish descent.

Enzyme defect and disease:

Glucocerebrosidase – Gaucher disease

Alpha-galactosidase A – Fabry disease

Galactosylceramidase – Krabbe disease

Hexosaminidase-Alpha unit – Tay-Sachs disease

Hexosaminidase-Beta unit – Sandhoff disease

Sphingomyelinase – Niemann-Pick disease

**208. B**

**Niemann-Pick disease**

Niemann-Pick disease is an autosomal recessive disorder that results from defects in acid sphingomyelinase. Type A has an early age of onset and progressive CNS disease. Type B has a more variable onset and progression of hepatosplenomegaly and pulmonary disease. Hepatic replacement by foam cells leads to cirrhosis.

**209. D**

Metastatic adenocarcinoma.

**210. B**

Epithelioid granuloma.

**211. B**

Metastatic melanoma (Black pigment is present)

**212. B**

Histoplasma

**213. A**

Pneumococcus

**214. C**

*Plasmodium falciparum* – “headphone” like structure

**215. B**

*Plasmodium vivax* – “ring” like structure

**216. A**

*Wuchereria bancrofti* and *Brugia malayi* are the two most common agents responsible for lymphatic filariasis. Both are thread-like worms that lie coiled in the lymphatic vessels and eventually reach the blood. Infection is mostly occurring in Asia (approximately 75% of cases),

Africa, Latin America, and the Pacific Islands. Definitive diagnosis requires the presence of microfilaria in the blood or lymphatic, ascitic, or pleural fluid.

Ehrlichiosis is a potentially life-threatening disease that is transmitted by ticks. Ehrlichia is a genus assigned to the family Rickettsiae. Two types of human ehrlichiosis have been identified in the United States: human monocytic ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE). The microorganisms undergo their life cycle in leukocytes and platelets. About half the patients present with mild leucopenia and thrombocytopenia. On peripheral blood smear, Ehrlichiosis shows cytoplasmic inclusion in monocytes and granulocytes.

**217. E**

This is a peripheral blood smear from a multiple myeloma patient, the blueish material around the red blood cell rouleaux formation is protein (monoclonal antibody or light chain). This high level circulating protein may cause significant hyperviscosity and may clog automated instrument or may results in falsely elevated white blood cell or platelet counts. Multiple myeloma may associated with Type I cryoglobulinemia, Hepatitis C viral infection is usually associated with type II or type III cryoglobulinemia.

**218. B**

IgD



# Appendix



## **WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues (2008; 4th Ed)**

---

### **MYELOPROLIFERATIVE NEOPLASMS**

- Chronic myelogenous leukemia, BCR-ABL1 positive
- Chronic neutrophilic leukemia
- Polycythemia vera
- Primary myelofibrosis
- Essential thrombocythemia
- Chronic eosinophilic leukemia, NOS
- Mastocytosis
  - Cutaneous mastocytosis
  - Systemic mastocytosis
  - Mast cell leukemia
  - Mast cell sarcoma
  - Extracutaneous mastocytoma
- Myeloproliferative neoplasm, unclassifiable

### **MYELOID AND LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND ABNORMALITIES OF PDGFRA, PDGFRB OR FGFR1**

- Myeloid and lymphoid neoplasms with PDGFRA rearrangement
- Myeloid neoplasms with PDGFRB rearrangement
- Myeloid and lymphoid neoplasms with FGFR1 abnormalities

### **MYELOYDYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS**

- Chronic myelomonocytic leukemia
- Atypical chronic myeloid leukemia, BCR-ABL1 negative
- Juvenile myelomonocytic leukemia
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable
- Refractory anemia with ring sideroblasts associated with marked thrombocytosis

### **MYELOYDYSPLASTIC SYNDROMES**

- Refractory cytopenia with unilineage dysplasia
- Refractory anemia

- Refractory neutropenia
- Refractory thrombocytopenia
- Refractory anemia with ring sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anemia with excess blasts
- Myelodysplastic syndrome associated with isolated del(5q)
- Myelodysplastic syndrome, unclassifiable
- Childhood myelodysplastic syndrome
- Refractory cytopenia of childhood

### **ACUTE MYELOID LEUKEMIA (AML) AND RELATED PRECURSOR NEOPLASMS**

- AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- Acute promyelocytic leukemia with t(15;17)(q22;q12); PML-RARA
- AML with t(9;11)(p22;q23); MLLT3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
- AML with mutated NPM1
- AML with mutated CEBPA

#### **AML with myelodysplasia-related changes**

#### **Therapy-related myeloid neoplasms**

### **ACUTE MYELOID LEUKEMIA, NOS**

- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

**Myeloid sarcoma**

**Myeloid proliferations related to Down syndrome**

- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

**Blastic plasmacytoid dendritic cell neoplasm**

**ACUTE LEUKEMIA OF AMBIGUOUS LINEAGE**

- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); BCR-ABL1
- Mixed phenotype acute leukemia with t(v;11 q23); MLL rearranged
- Mixed phenotype acute leukemia, B/myeloid, NOS
- Mixed phenotype acute leukemia, T/myeloid, NOS
- Natural killer (NK) cell lymphoblastic leukemia/lymphoma

**PRECURSOR LYMPHOID NEOPLASMS**

**B lymphoblastic leukemia/lymphoma**

- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2);BCR ABL1
- B lymphoblastic leukemia/lymphoma with t(v;11q23); MLL rearranged
- B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); TEL-AML1(ETV6-RUNX1)
- B lymphoblastic leukemia/lymphoma with hyperdiploidy
- B lymphoblastic leukemia/lymphoma with hypodiploidy (hypodiploid ALL)
- B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32);L3-/GH
- B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); E2A-PBX1(TCF3-PBX1)

**T lymphoblastic leukemia/lymphoma**

**MATURE B-CELL NEOPLASMS**

- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Splenic B-cell marginal zone lymphoma
- Hairy cell leukemia
  - Splenic B-cell lymphoma/leukemia, unclassifiable
  - Splenic diffuse red pulp small B-cell lymphoma
- Lymphoplasmacytic lymphoma
  - Waldenström macroglobulinemia

- Heavy chain diseases
  - Alpha heavy chain disease
  - Gamma heavy chain disease
  - Mu heavy chain disease
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
  - Pediatric nodal marginal zone lymphoma
- Follicular lymphoma
  - Pediatric follicular lymphoma
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma (DLBCL), NOS
  - T-cell/histiocyte rich large B-cell lymphoma
  - Primary DLBCL of the CNS
  - Primary cutaneous DLBCL, leg type
  - EBV positive DLBCL of the elderly
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK positive large B-cell lymphoma
- Plasmablastic lymphoma
- Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
- Primary effusion lymphoma
- Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

#### **MATURE T-CELL AND NK-CELL NEOPLASMS**

- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorder of NK-cells



- Aggressive NK cell leukemia
- Systemic EBV positive T-cell lymphoproliferative disease of childhood
- Hydroa vacciniforme-like lymphoma
- Adult T-cell leukemia/lymphoma
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
- Lymphomatoid papulosis
- Primary cutaneous anaplastic large cell lymphoma
- Primary cutaneous gamma-delta T-cell lymphoma
- Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
- Primary cutaneous CD4 positive small/medium T-cell lymphoma
- Peripheral T-cell lymphoma, NOS
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, ALK positive
- Anaplastic large cell lymphoma, ALK negative

### **HODGKIN LYMPHOMA**

- Nodular lymphocyte predominant Hodgkin lymphoma
- Classical Hodgkin lymphoma
  - Nodular sclerosis classical Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Mixed cellularity classical Hodgkin lymphoma
  - Lymphocyte-depleted classical Hodgkin lymphoma

### **HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS**

- Histiocytic sarcoma
- Langerhans cell histiocytosis
- Langerhans cell sarcoma
- Interdigitating dendritic cell sarcoma
- Follicular dendritic cell sarcoma
- Fibroblastic reticular cell tumor

- Indeterminate dendritic cell tumor
- Disseminated juvenile xanthogranuloma

### **POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD)**

- Early lesions
  - Plasmacytic hyperplasia
  - Infectious mononucleosis-like PTLD
- Polymorphic PTLD
- Monomorphic PTLD (B- and T/NK-cell types)
- Classical Hodgkin lymphoma type PTLD

**\*NOS, not otherwise specified**

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